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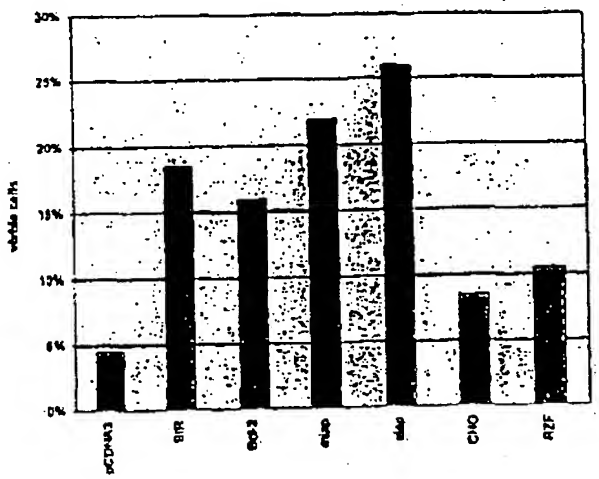

BIR = BACULOVIRUS IAP REPEAT
RZF = RING ZINC FINGER

(57) Abstract

Disclosed is substantially pure DNA encoding mammalian IAP polypeptides; substantially pure polypeptides; and methods of using such DNA to express the IAP polypeptides in cells and animals to inhibit apoptosis. Also disclosed are conserved regions characteristic of the IAP family and primers and probes for the identification and isolation of additional IAP genes. In addition, methods for treating diseases and disorders involving apoptosis are provided.

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MAMMALIAN APOPTOSIS INHIBITOR PROTEIN GENE FAMILY, PRIMERS, PROBES AND DETECTION METHOD

Background of the Invention

The invention relates to apoptosis.

There are two general ways by which cells die. The most easily recognized way is by necrosis, which is usually caused by an injury that is severe enough to disrupt cellular homeostasis. Typically, the cell's osmotic pressure is disturbed and, consequently, the cell swells and then ruptures. When the cellular contents are spilled into the surrounding tissue space, an inflammatory response often ensues.

The second general way by which cells die is referred to as apoptosis, or programmed cell death. Apoptosis often occurs so rapidly that it is difficult to detect. This may help to explain why the involvement of apoptosis in a wide spectrum of biological processes has only recently been recognized.

The apoptosis pathway has been highly conserved throughout evolution, and plays a critical role in embryonic development, viral pathogenesis, cancer, autoimmune disorders, and neurodegenerative disease. For example, inappropriate apoptosis may cause or contribute to AIDS, Alzheimer's Disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis (ALS), retinitis pigmentosa and other diseases of the retina, myelodysplastic syndrome (e.g. aplastic anemia), toxin-induced liver disease, including alcoholism, and ischemic injury (e.g. myocardial infarction, stroke, and reperfusion injury). Conversely, the failure of an apoptotic response has been implicated in the development of cancer, particularly follicular lymphoma, p53-mediated carcinomas, and hormone-dependent tumors, in autoimmune disorders, such as lupus erythematosis and multiple

sclerosis, and in viral infections, including those associated with herpes virus, poxvirus, and adenovirus.

In patients infected with HIV-1, mature $CD4^+$ T lymphocytes respond to stimulation from mitogens or super-antigens by undergoing apoptosis. However, the great majority of these cells are not infected with the virus. Thus, inappropriate antigen-induced apoptosis could be responsible for the destruction of this vital part of the immune system in the early stages of HIV infection.

Baculoviruses encode proteins that are termed inhibitors of apoptosis proteins (IAPs) because they inhibit the apoptosis that would otherwise occur when insect cells are infected by the virus. These proteins are thought to work in a manner that is independent of other viral proteins. The baculovirus IAP genes include sequences encoding a ring zinc finger-like motif (RZF), which is presumed to be directly involved in DNA binding, and two N-terminal domains that consist of a 70 amino acid repeat motif termed a BIR domain (Baculovirus IAP Repeat).

Summary of the Invention

In general, the invention features a substantially pure DNA molecule, such as a genomic, cDNA, or synthetic DNA molecule, that encodes a mammalian IAP polypeptide. This DNA may be incorporated into a vector, into a cell, which may be a mammalian, yeast, or bacterial cell, or into a transgenic animal or embryo thereof. In preferred embodiments, the DNA molecule is a murine gene (e.g., m-xiap, m-hiap-1, or m-hiap-2) or a human gene (e.g., xiap, hiap-1, or hiap-2). In most preferred embodiments the IAP gene is a human IAP gene. In other various preferred embodiments, the cell is a transformed cell. In related aspects, the invention features a transgenic animal

containing a transgene that encodes an IAP polypeptide that is expressed in or delivered to tissue normally susceptible to apoptosis, i.e., to a tissue that may be harmed by either the induction or repression of apoptosis. In yet another aspect, the invention features DNA encoding fragments of IAP polypeptides including the BIR domains and the RZF domains provided herein.

In specific embodiments, the invention features DNA sequences substantially identical to the DNA sequences shown in Figs. 1-6, or fragments thereof. In another aspect, the invention also features RNA which is encoded by the DNA described herein. Preferably, the RNA is mRNA. In another embodiment the RNA is antisense RNA.

In another aspect, the invention features a substantially pure polypeptide having a sequence substantially identical to one of the IAP amino acid sequences shown in Figures 1-6.

In a second aspect, the invention features a substantially pure DNA which includes a promoter capable of expressing the IAP gene in a cell susceptible to apoptosis. In preferred embodiments, the IAP gene is xiap, hiap-1, or hiap-2. Most preferably, the genes are human or mouse genes. The gene encoding hiap-2 may be the full-length gene, as shown in Fig. 3, or a truncated variant, such as a variant having a deletion of the sequence boxed in Fig. 3.

In preferred embodiments, the promoter is the promoter native to an IAP gene. Additionally, transcriptional and translational regulatory regions are, preferably, those native to an IAP gene. In another aspect, the invention provides transgenic cell lines and transgenic animals. The transgenic cells of the invention are preferably cells that are altered in their apoptotic response. In preferred embodiments, the transgenic cell is a fibroblast, neuronal cell, a

lymphocyte cell, a glial cell, an embryonic stem cell, or an insect cell. Most preferably, the neuron is a motor neuron and the lymphocyte is a $CD4^+$ T cell.

In another aspect, the invention features a method of inhibiting apoptosis that involves producing a transgenic cell having a transgene encoding an IAP polypeptide. The transgene is integrated into the genome of the cell in a way that allows for expression. Furthermore, the level of expression in the cell is sufficient to inhibit apoptosis.

In a related aspect, the invention features a transgenic animal, preferably a mammal, more preferably a rodent, and most preferably a mouse, having either increased copies of at least one IAP gene inserted into the genome (mutant or wild-type), or a knockout of at least one IAP gene in the genome. The transgenic animals will express either an increased or a decreased amount of IAP polypeptide, depending on the construct used and the nature of the genomic alteration. For example, utilizing a nucleic acid molecule that encodes all or part of an IAP to engineer a knockout mutation in an IAP gene would generate an animal with decreased expression of either all or part of the corresponding IAP polypeptide. In contrast, inserting exogenous copies of all or part of an IAP gene into the genome, preferably under the control of active regulatory and promoter elements, would lead to increased expression or the corresponding IAP polypeptide.

In another aspect, the invention features a method of detecting an IAP gene in a cell by contacting the IAP gene, or a portion thereof (which is greater than 9 nucleotides, and preferably greater than 18 nucleotides in length), with a preparation of genomic DNA from the cell. The IAP gene and the genomic DNA are brought into contact under conditions that allow for hybridization

(and therefore, detection) of DNA sequences in the cell that are at least 50% identical to the DNA encoding HIAP-1, HIAP-2, or XIAP polypeptides.

In another aspect, the invention features a method of producing an IAP polypeptide. This method involves providing a cell with DNA encoding all or part of an IAP polypeptide (which is positioned for expression in the cell), culturing the cell under conditions that allow for expression of the DNA, and isolating the IAP polypeptide. In preferred embodiments, the IAP polypeptide is expressed by DNA that is under the control of a constitutive or inducible promotor. As described herein, the promotor may be a heterologous promotor.

In another aspect, the invention features substantially pure mammalian IAP polypeptide. Preferably, the polypeptide includes an amino acid sequence that is substantially identical to all, or to a fragment of, the amino acid sequence shown in any one of Figs. 1-4. Most preferably, the polypeptide is the XIAP, HIAP-1, HIAP-2, M-XIAP, M-HIAP-1, or M-HIAP-2 polypeptide. Fragments including one or more BIR domains (to the exclusion of the RZF), the RZF domain (to the exclusion of the BIR domains), and a RZF domain with at least one BIR domain, as provided herein, are also a part of the invention.

In another aspect, the invention features a recombinant mammalian polypeptide that is capable of modulating apoptosis. The polypeptide may include at least a ring zinc finger domain and a BIR domain as defined herein. In preferred embodiments, the invention features (a) a substantially pure polypeptide, and (b) an oligonucleotide encoding the polypeptide. In instances were the polypeptide includes a ring zinc finger domain, the ring zinc finger domain will have a sequence conforming to: Glu-Xaa1-Xaa1-Xaa1- Xaa1-Xaa1-Xaa1-Xaa2-

Xaa1-Xaa1-Xaa1-Cys-Lys-Xaa3-Cys-Met-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa3-Xaa1-Phe-Xaa1-Pro-Cys-Gly-His-Xaa1-Xaa1-Xaa1-Cys-Xaa1-Xaa1-Cys-Ala-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Cys-Pro-Xaa1-Cys, where Xaa1 is any amino acid, Xaa2 is Glu or Asp, Xaa3 is Val or Ile (SEQ ID NO:1); and where the polypeptide includes at least one BIR domain, the BIR domain will have a sequence conforming to: Xaa1-Xaa1-Xaa1-Arg-Leu-Xaa1-Thr-Phe-Xaa1-Xaa1-Trp-Pro-Xaa2-Xaa1-Xaa1-Xaa2-Xaa2-Xaa1-Xaa1-Xaa1-Xaa1-Leu-Ala-Xaa1-Ala-Gly-Phe-Tyr-Tyr-Xaa1-Gly-Xaa1-Xaa1-Asp-Xaa1-Val-Xaa1-Cys-Phe-Xaa1-Cys-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Trp-Xaa1-Xaa1-Xaa1-Asp-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-His-Xaa1-Xaa1-Xaa1-Xaa1-Pro-Xaa1-Cys-Xaa1-Phe-Val, where Xaa1 may be any amino acid and Xaa2 may be any amino acid or may be absent (SEQ ID NO:2).

In various preferred embodiments the polypeptide has at least two or, more preferably at least three BIR domains, the RZF domain has one of the IAP sequences shown in Fig. 6, and the BIR domains are comprised of BIR domains shown in Fig. 5. In other preferred embodiments the BIR domains are at the amino terminal end of the protein relative to the RZF domain, which is at or near the carboxyl terminus of the polypeptide.

In another aspect, the invention features an IAP gene isolated according to the method involving: (a) providing a sample of DNA; (b) providing a pair of oligonucleotides having sequence homology to a conserved region of an IAP disease-resistance gene; (c) combining the pair of oligonucleotides with the cell DNA sample under conditions suitable for polymerase chain reaction-mediated DNA amplification; and (d) isolating the amplified IAP gene or fragment thereof.

In preferred embodiments, the amplification is carried out using a reverse-transcription polymerase chain reaction, for example, the RACE method. In another

aspect, the invention features an IAP gene isolated according to the method involving: (a) providing a preparation of DNA; (b) providing a detectably labelled DNA sequence having homology to a conserved region of an IAP gene; (c) contacting the preparation of DNA with the detectably-labelled DNA sequence under hybridization conditions providing detection of genes having 50% or greater nucleotide sequence identity; and (d) identifying an IAP gene by its association with the detectable label.

In another aspect, the invention features an IAP gene isolated according to the method involving: (a) providing a cell sample; (b) introducing by transformation into the cell sample a candidate IAP gene; (c) expressing the candidate IAP gene within the cell sample; and (d) determining whether the cell sample exhibits an altered apoptotic response, whereby a response identifies an IAP gene.

In another aspect, the invention features a method of identifying an IAP gene in a cell, involving: (a) providing a preparation of cellular DNA (for example, from the human genome or a cDNA library (such as a cDNA library isolated from a cell type which undergoes apoptosis); (b) providing a detectably-labelled DNA sequence (for example, prepared by the methods of the invention) having homology to a conserved region of an IAP gene; (c) contacting the preparation of cellular DNA with the detectably-labelled DNA sequence under hybridization conditions providing detection of genes having 50% nucleotide or greater sequence identity; and (d) identifying an IAP gene by its association with the detectable label.

In another aspect, the invention features a method of isolating an IAP gene from a recombinant library, involving: (a) providing a recombinant library; (b) contacting the library with a detectably-labelled

gene fragment produced according to the PCR method of the invention under hybridization conditions providing detection of genes having 50% or greater nucleotide sequence identity; and (c) isolating an IAP gene by its association with the detectable label. In another aspect, the invention features a method of identifying an IAP gene involving: (a) providing a cell tissue sample; (b) introducing by transformation into the cell sample a candidate IAP gene; (c) expressing the candidate IAP gene within the cell sample; and (d) determining whether the cell sample exhibits inhibition of apoptosis, whereby a change in (i.e. modulation of) apoptosis identifies an IAP gene. Preferably, the cell sample is a cell type that may be assayed for apoptosis (e.g., T cells, B cells, neuronal cells, baculovirus-infected insect cells, glial cells, embryonic stem cells, and fibroblasts). The candidate IAP gene is obtained, for example, from a cDNA expression library, and the response assayed is the inhibition of apoptosis.

In another aspect, the invention features a method of inhibiting apoptosis in a mammal wherein the method includes: (a) providing DNA encoding at least one IAP polypeptide to a cell that is susceptible to apoptosis; wherein the DNA is integrated into the genome of the cell and is positioned for expression in the cell; and the IAP gene is under the control of regulatory sequences suitable for controlled expression of the gene(s); wherein the IAP transgene is expressed at a level sufficient to inhibit apoptosis relative to a cell lacking the IAP transgene. The DNA integrated into the genome may encode all or part of an IAP polypeptide. It may, for example, encode a ring zinc finger and one or more BIR domains. In contrast, it may encode either the ring zinc finger alone, or one or more BIR domains alone. Skilled artisans will appreciate that IAP polypeptides

may also be administered directly to inhibit undesirable apoptosis.

In a related aspect, the invention features a method of inhibiting apoptosis by producing a cell that has integrated, into its genome, a transgene that includes the IAP gene, or a fragment thereof. The IAP gene may be placed under the control of a promoter providing constitutive expression of the IAP gene. Alternatively, the IAP transgene may be placed under the control of a promoter that allows expression of the gene to be regulated by environmental stimuli. For example, the IAP gene may be expressed using a tissue-specific or cell type-specific promoter, or by a promoter that is activated by the introduction of an external signal or agent, such as a chemical signal or agent. In preferred embodiments the cell is a lymphocyte, a neuronal cell, a glial cell, or a fibroblast. In other embodiments, the cell in an HIV-infected human, or in a mammal suffering from a neurodegenerative disease, an ischemic injury, a toxin-induced liver disease, or a myelodysplastic syndrome.

In a related aspect, the invention provides a method of inhibiting apoptosis in a mammal by providing an apoptosis-inhibiting amount of IAP polypeptide. The IAP polypeptide may be a full-length polypeptide, or it may be one of the fragments described herein.

In another aspect, the invention features a purified antibody that binds specifically to an IAP family protein. Such an antibody may be used in any standard immunodetection method for the identification of an IAP polypeptide. Preferably, the antibody binds specifically to XIAP, HIAP-1, or HIAP-2. In various embodiments, the antibody may react with other IAP polypeptides or may be specific for one or a few IAP polypeptides. The antibody may be a monoclonal or a

polyclonal antibody. Preferably, the antibody reacts specifically with only one of the IAP polypeptides, for example, reacts with murine and human xiap, but not with hiap-1 or hiap-2 from other mammalian species.

The antibodies of the invention may be prepared by a variety of methods. For example, the IAP polypeptide, or antigenic fragments thereof, can be administered to an animal in order to induce the production of polyclonal antibodies. Alternatively, antibodies used as described herein may be monoclonal antibodies, which are prepared using hybridoma technology (see, e.g., Kohler et al., Nature 256:495, 1975; Kohler et al., Eur. J. Immunol. 6:511, 1976; Kohler et al., Eur. J. Immunol. 6:292, 1976; Hammerling et al., In Monoclonal Antibodies and T Cell Hybridomas, Elsevier, NY, 1981). The invention features antibodies that specifically bind human or murine IAP polypeptides, or fragments thereof. In particular the invention features "neutralizing" antibodies. By "neutralizing" antibodies is meant antibodies that interfere with any of the biological activities of IAP polypeptides, particularly the ability of IAPs to inhibit apoptosis. The neutralizing antibody may reduce the ability of IAP polypeptides to inhibit polypeptides by, preferably 50%, more perferably by 70, and most preferably by 90% or more. Any standard assay of apoptosis, including those described herein, may be used to assess neutralizing antibodies.

In addition to intact monoclonal and polyclonal anti-IAP antibodies, the invention features various genetically engineered antibodies, humanized antibodies, and antibody fragments, including F(ab')2, Fab', Fab, Fv and sFv fragments. Antibodies can be humanized by methods known in the art, e.g., monoclonal antibodies with a desired binding specificity can be commercially humanized (Scotgene, Scotland; Oxford Molecular, Palo

Alto, CA). Fully human antibodies, such as those expressed in transgenic animals, are also features of the invention (Green et al., Nature Genetics 7:13-21, 1994).

Ladner (U.S. Patent 4,946,778 and 4,704,692) describes methods for preparing single polypeptide chain antibodies. Ward et al. (Nature 341:544-546, 1989) describe the preparation of heavy chain variable domains, which they term "single domain antibodies," which have high antigen-binding affinities. McCafferty et al. (Nature 348:552-554, 1990) show that complete antibody V domains can be displayed on the surface of fd bacteriophage, that the phage bind specifically to antigen, and that rare phage (one in a million) can be isolated after affinity chromatography. Boss et al. (U.S. Patent 4,816,397) describe various methods for producing immunoglobulines, and immunologically functional fragments thereof, which include at least the variable domains of the heavy and light chain in a single host cell. Cabilly et al. (U.S. Patent 4,816,567) describe methods for preparing chimeric antibodies.

In another aspect, the invention features a method of identifying a compound that modulates apoptosis. The method includes providing a cell expressing an IAP polypeptide, contacting the cell with a candidate compound, and monitoring the expression of an IAP gene. An alteration in the level of expression of the IAP gene indicates the presence of a compound which modulates apoptosis. The compound may be an inhibitor or an enhancer of apoptosis. In various preferred embodiments, the cell is a fibroblast, a neuronal cell, a glial cell, a lymphocyte (T cell or B cell), or an insect cell; the polypeptide expression being monitored is XIAP, HIAP-1, HIAP-2, M-XIAP, M-HIAP-1, or M-HIAP-2 (i.e., human or murine).

In a related aspect, the invention features methods of detecting compounds that modulate apoptosis using the interaction trap technology and IAP polypeptides, or fragments thereof, as a component of the bait. In preferred embodiments, the compound being tested as a modulator of apoptosis is also a polypeptide.

In another aspect, the invention features a method for diagnosing a cell proliferation disease, or an increased likelihood of such a disease, using an IAP nucleic acid probe or antibody. Preferably, the disease is a cancer. Most preferably, the disease is selected from the group consisting of promyelocytic leukemia, a HeLa-type carcinoma, chronic myelogenous leukemia (preferably using xiap or hiap-2 related probes), lymphoblastic leukemia (preferably using a xiap related probe), Burkitt's lymphoma (preferably using an hiap-1 related probe), colorectal adenocarcinoma, lung carcinoma, and melanoma (preferably using a xiap probe). Preferably, a diagnosis is indicated by a 2-fold increase in expression or activity, more preferably, at least a 10-fold increase in expression or activity.

Skilled artisans will recognize that a mammalian IAP, or a fragment thereof (as described herein), may serve as an active ingredient in a therapeutic composition. This composition, depending on the IAP or fragment included, may be used to modulate apoptosis and thereby treat any condition that is caused by a disturbance in apoptosis.

In addition, apoptosis may be induced in a cell by administering to the cell a negative regulator of the IAP-dependent anti-apoptotic pathway. The negative regulator may be, but is not limited to, an IAP polypeptide that includes a ring zinc finger, and an IAP polypeptide that includes a ring zinc finger and lacks at least one BIR domain. Alternatively, apoptosis may be

induced in the cell by administering a gene encoding an IAP polypeptide, such as these two polypeptides. In yet another method, the negative regulator may be a purified antibody, or a fragment thereof, that binds specifically to an IAP polypeptide. For example, the antibody may bind to an approximately 26 kDa cleavage product of an IAP polypeptide that includes at least one BIR domain but lacks a ring zinc finger domain. The negative regulator may also be an IAP antisense mRNA molecule.

As summarized above, an IAP nucleic acid, or an IAP polypeptide may be used to modulate apoptosis. Furthermore, an IAP nucleic acid, or an IAP polypeptide, may be used in the manufacture of a medicament for the modulation of apoptosis.

By "IAP gene" is meant a gene encoding a polypeptide having at least one BIR domain and a ring zinc finger domain which is capable of modulating (inhibiting or enhancing) apoptosis in a cell or tissue when provided by other intracellular or extracellular delivery methods. In preferred embodiments the IAP gene is a gene having about 50% or greater nucleotide sequence identity to at least one of the IAP amino acid encoding sequences of Figs. 1-4 or portions thereof. Preferably, the region of sequence over which identity is measured is a region encoding at least one BIR domain and a ring zinc finger domain. Mammalian IAP genes include nucleotide sequences isolated from any mammalian source. Preferably, the mammal is a human.

The term "IAP gene" is meant to encompass any member of the family of apoptosis inhibitory genes, which are characterized by their ability to modulate apoptosis. An IAP gene may encode a polypeptide that has at least 20%, preferably at least 30%, and most preferably at least 50% amino acid sequence identity with at least one of the conserved regions of one of the IAP members

described herein (i.e., either the BIR or ring zinc finger domains from the human or murine xiap, hiap-1 and hiap-2). Representative members of the IAP gene family include, without limitation, the human and murine xiap, hiap-1, and hiap-2 genes.

By "IAP protein" or "IAP polypeptide" is meant a polypeptide, or fragment thereof, encoded by an IAP gene.

By "BIR domain" is meant a domain having the amino acid sequence of the consensus sequence: Xaa1-Xaa1-Xaa1-Arg-Leu-Xaa1-Thr-Phe-Xaa1-Xaa1-Trp-Pro-Xaa2-Xaa1-Xaa1-Xaa2-Xaa2-Xaa1-Xaa1-Xaa1-Xaa1-Leu-Ala-Xaa1-Ala-Gly-Phe-Tyr-Tyr-Xaa1-Gly-Xaa1-Xaa1-Asp-Xaa1-Val-Xaa1-Cys-Phe-Xaa1-Cys-Xaa1-Xaa1- Xaa1-Xaa1-Xaa1-Xaa1-Trp-Xaa1-Xaa1-Xaa1-Asp-Xaa1-Xaa1-Xaa1- Xaa1-Xaa1-His-Xaa1-Xaa1-Xaa1-Xaa1-Pro-Xaa1-Cys-Xaa1-Phe-Val, wherein Xaa1 is any amino acid and Xaa2 is any amino acid or is absent (SEQ ID NO:2). Preferably, the sequence is substantially identical to one of the BIR domain sequences provided for xiap, hiap-1, hiap-2 herein.

By "ring zinc finger" or "RZF" is meant a domain having the amino acid sequence of the consensus sequence: Glu-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa2-Xaa1-Xaa1-Xaa1-Cys- Lys-Xaa3-Cys-Met-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa3-Xaa1-Phe-Xaa1-Pro-Cys-Gly-His-Xaa1-Xaa1-Xaa1-Cys-Xaa1-Xaa1-Cys-Ala- Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Cys-Pro-Xaa1-Cys, wherein Xaa1 is any amino acid, Xaa2 is Glu or Asp, and Xaa3 is Val or Ile (SEQ ID NO:1).

Preferably, the sequence is substantially identical to the RZF domains provided herein for the human or murine xiap, hiap-1, or hiap-2.

By "modulating apoptosis" or "altering apoptosis" is meant increasing or decreasing the number of cells that would otherwise undergo apoptosis in a given cell population. Preferably, the cell population is selected from a group including T cells, neuronal

cells, fibroblasts, or any other cell line known to undergo apoptosis in a laboratory setting (e.g., the baculovirus infected insect cells). It will be appreciated that the degree of modulation provided by an IAP or modulating compound in a given assay will vary, but that one skilled in the art can determine the statistically significant change in the level of apoptosis which identifies an IAP or a compound which modulates an IAP.

By "inhibiting apoptosis" is meant any decrease in the number of cells which undergo apoptosis relative to an untreated control. Preferably, the decrease is at least 25%, more preferably the decrease is 50%, and most preferably the decrease is at least one-fold.

By "polypeptide" is meant any chain of more than two amino acids, regardless of post-translational modification such as glycosylation or phosphorylation.

By "substantially identical" is meant a polypeptide or nucleic acid exhibiting at least 50%, preferably 85%, more preferably 90%, and most preferably 95% homology to a reference amino acid or nucleic acid sequence. For polypeptides, the length of comparison sequences will generally be at least 16 amino acids, preferably at least 20 amino acids, more preferably at least 25 amino acids, and most preferably 35 amino acids. For nucleic acids, the length of comparison sequences will generally be at least 50 nucleotides, preferably at least 60 nucleotides, more preferably at least 75 nucleotides, and most preferably 110 nucleotides.

Sequence identity is typically measured using sequence analysis software with the default parameters specified therein (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). This software program matches

similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine, valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

By "substantially pure polypeptide" is meant a polypeptide that has been separated from the components that naturally accompany it. Typically, the polypeptide is substantially pure when it is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the polypeptide is an IAP polypeptide that is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, pure. A substantially pure IAP polypeptide may be obtained, for example, by extraction from a natural source (e.g. a fibroblast, neuronal cell, or lymphocyte) by expression of a recombinant nucleic acid encoding an IAP polypeptide, or by chemically synthesizing the protein. Purity can be measured by any appropriate method, e.g., by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

A protein is substantially free of naturally associated components when it is separated from those contaminants which accompany it in its natural state. Thus, a protein which is chemically synthesized or produced in acellular system different from the cell from which it naturally originates will be substantially free from its naturally associated components. Accordingly, substantially pure polypeptides include those derived from eukaryotic organisms but synthesized in *E. coli* or other prokaryotes. By "substantially pure DNA" is meant DNA that is free of the genes which, in the naturally-

occurring genome of the organism from which the DNA of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or which exists as a separate molecule (e.g., a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence.

By "transformed cell" is meant a cell into which (or into an ancestor of which) has been introduced, by means of recombinant DNA techniques, a DNA molecule encoding (as used herein) an IAP polypeptide.

By "transgene" is meant any piece of DNA which is inserted by artifice into a cell, and becomes part of the genome of the organism which develops from that cell. Such a transgene may include a gene which is partly or entirely heterologous (i.e., foreign) to the transgenic organism, or may represent a gene homologous to an endogenous gene of the organism.

By "transgenic" is meant any cell which includes a DNA sequence which is inserted by artifice into a cell and becomes part of the genome of the organism which develops from that cell. As used herein, the transgenic organisms are generally transgenic mammalian (e.g., rodents such as rats or mice) and the DNA (transgene) is inserted by artifice into the nuclear genome.

By "transformation" is meant any method for introducing foreign molecules into a cell. Lipofection, calcium phosphate precipitation, retroviral delivery, electroporation, and biolistic transformation are just a few of the teachings which may be used. For example, biolistic transformation is a method for introducing

foreign molecules into a cell using velocity driven microprojectiles such as tungsten or gold particles. Such velocity-driven methods originate from pressure bursts which include, but are not limited to, helium-driven, air-driven, and gunpowder-driven techniques. Biolistic transformation may be applied to the transformation or transfection of a wide variety of cell types and intact tissues including, without limitation, intracellular organelles (e.g., and mitochondria and chloroplasts), bacteria, yeast, fungi, algae, animal tissue, and cultured cells.

By "positioned for expression" is meant that the DNA molecule is positioned adjacent to a DNA sequence which directs transcription and translation of the sequence (i.e., facilitates the production of, e.g., an IAP polypeptide, a recombinant protein or a RNA molecule).

By "reporter gene" is meant a gene whose expression may be assayed; such genes include, without limitation, glucuronidase (GUS), luciferase, chloramphenicol transacetylase (CAT), and β-galactosidase.

By "promoter" is meant minimal sequence sufficient to direct transcription. Also included in the invention are those promoter elements which are sufficient to render promoter-dependent gene expression controllable for cell type-specific, tissue-specific or inducible by external signals or agents; such elements may be located in the 5′ or 3′ regions of the native gene.

By "operably linked" is meant that a gene and one or more regulatory sequences are connected in such a way as to permit gene expression when the appropriate molecules (e.g., transcriptional activator proteins are bound to the regulatory sequences).

By "conserved region" is meant any stretch of six or more contiguous amino acids exhibiting at least 30%, preferably 50%, and most preferably 70% amino acid sequence identity between two or more of the IAP family members, (e.g., between human HIAP-1, HIAP-2, and XIAP). Examples of preferred conserved regions are shown (as boxed or designated sequences) in Figures 5-7 and Tables 1 and 2, and include, without limitation, BIR domains and ring zinc finger domains.

By "detectably-labelled" is meant any means for marking and identifying the presence of a molecule, e.g., an oligonucleotide probe or primer, a gene or fragment thereof, or a cDNA molecule. Methods for detectably-labelling a molecule are well known in the art and include, without limitation, radioactive labelling (e.g., with an isotope such as ^{32}P or ^{35}S) and nonradioactive labelling (e.g., chemiluminescent labelling, e.g., fluorescein labelling).

By "antisense," as used herein in reference to nucleic acids, is meant a nucleic acid sequence, regardless of length, that is complementary to the coding strand of a gene.

By "purified antibody" is meant antibody which is at least 60%, by weight, free from proteins and naturallyoccurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably 90%, and most preferably at least 99%, by weight, antibody, e.g., an IAP specific antibody. A purified antibody may be obtained, for example, by affinity chromatography using recombinantly-produced protein or conserved motif peptides and standard techniques.

By "specifically binds" is meant an antibody that recognizes and binds a protein but that does not substantially recognize and bind other molecules in a

sample, e.g., a biological sample, that naturally includes protein.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Brief Description of the Drawings

Fig. 1 is the human xiap cDNA sequence (SEQ ID NO:3) and the XIAP polypeptide sequence (SEQ ID NO:4).

Fig. 2 is the human hiap-1 cDNA sequence (SEQ ID NO:5) and the HIAP-1 polypeptide sequence (SEQ ID NO:6).

Fig. 3 is the human hiap-2 cDNA sequence (SEQ ID NO:7) and the HIAP-2 polypeptide sequence (SEQ ID NO:8). The sequence absent in the hiap-2-Δ variant is boxed.

Fig. 4 is the murine xiap cDNA sequence (SEQ ID NO:9) and encoded murine XIAP polypeptide sequence (SEQ ID NO:10).

Fig. 5 is the murine hiap-1 cDNA sequence (SEQ ID NO:39) and the encoded murine HIAP-1 polypeptide sequence (SEQ ID NO:40).

Fig. 6 is the murine hiap-2 cDNA sequence (SEQ ID NO:41) and the encoded murine HIAP-2 polypeptide (SEQ ID NO:42).

Fig. 7 is a representation of the alignment of the BIR domains of IAP proteins (SEQ ID NOs 11 and 14-31).

Fig. 8 is a representation of the alignment of human IAP polypeptides with diap, cp-iap, and the IAP consensus sequence (SEQ ID NOs:4, 6, 8, 10, 12, and 13).

Fig. 9 is a representation of the alignment of the ring zinc finger domains of IAP proteins (SEQ ID NOs:32-38).

Fig. 10 is a photograph of a Northern blot illustrating human hiap-1 and hiap-2 mRNA expression in human tissues.

Fig. 11 is a photograph of a Northern blot illustrating human hiap-2 mRNA expression in human tissues.

Fig. 12 is a photograph of a Northern blot illustrating human xiap mRNA expression in human tissues.

Fig. 13A and 13B are photographs of agarose gels illustrating apoptotic DNA ladders and RT-PCR products using hiap-1 and hiap-2 specific probes in HIV-infected T cells.

Fig. 14A - 14D are graphs depicting suppression of apoptosis by XIAP, HIAP-1, HIAP-2, bcl-2, smn, and 6-myc.

Fig. 15A - 15B are bar graphs depicting the percentage of viable CHO cells following transient transfection with the cDNA constructs shown and subsequent serum withdrawal.

Fig. 16A - 16B are bar graphs depicting the percentage of viable CHO cells following transient transfection with the cDNA constructs shown and subsequent exposure to menadione (Fig. 16A = 10 μM menadione; Fig. 16B = 20 μM menadione).

Fig. 17 is a photograph of an agarose gel containing cDNA fragments that were amplified, with hiap-1-specific primers, from RNA obtained from Raji, Ramos, EB-3, and Jiyoye cells, and from normal placenta.

Fig. 18 is a photograph of a Western blot containing protein extracted from Jurkat and astrocytoma cells stained with an anti-XIAP antibody. The position and size of a series of marker proteins is indicated.

Fig. 19 is a photograph of a Western blot containing protein extracted from Jurkat cells following treatment as described in Example XII. The blot was

stained with a rabbit polyclonal anti-XIAP antibody. Lane 1, negative control; lane 2, anti-Fas antibody; lane 3, anti-Fas antibody and cycloheximide; lane 4, TNF-α; lane 5, TNF-α and cycloheximide.

Fig. 20 is a photograph of a Western blot containing protein extracted from HeLa cells following exposure to anti-Fas antibodies. The blot was stained with a rabbit polyclonal anti-XIAP antibody. Lane 1, negative control; lane 2, cycloheximide; lane 3, anti-Fas antibody; lane 4, anti-Fas antibody and cycloheximide; lane 5, TNF-α; lane 6, TNF-α and cycloheximide.

Fig. 21A - 21B are photographs of Western blots stained with rabbit polyclonal anti-XIAP antibody. Protein was extracted from HeLa cells (Fig. 21A) and Jurkat cells (Fig. 21B) immediately, 1, 2, 3, 5, 10, and 22 hours after exposure to anti-Fas antibody.

Fig. 22A and 22B are photographs of Western blots stained with an anti-CPP32 antibody (Fig. 22A) or a rabbit polyclonal anti-XIAP antibody (Fig. 22B). Protein was extracted from Jurkat cells immediately, 3 hours, or 7 hours after exposure to an anti-Fas antibody. In addition to total protein, cytoplasmic and nuclear extracts are shown.

Fig. 23 is a photograph of a polyacrylamide gel following electrophoresis of the products of an *in vitro* XIAP cleavage assay.

Detailed Description

I. IAP Genes and Polypeptides

A new class of mammalian proteins that modulate apoptosis (IAPS) and the genes that encode these proteins have been discovered. The IAP proteins are characterized by the presence of a ring zinc finger domain (RZF; Fig. 9) and at least one BIR domain, as defined by the boxed consensus sequences shown in Figs. 7 and 8, and by the

sequence domains listed in Tables 1 and 2. As examples of novel IAP genes and proteins, the cDNA sequences and amino acid sequences for human IAPs (HIAP-1, HIAP-2, and XIAP) and a new murine inhibitor of apoptosis, XIAP, are provided. Additional members of the mammalian IAP family (including homologs from other species and mutant sequences) may be isolated using standard cloning techniques and the conserved amino acid sequences, primers, and probes provided herein and known in the art. Furthermore, IAPs include those proteins lacking the ring zinc finger, as further described below.

TABLE 1

NUCLEOTIDE POSITION OF CONSERVED DOMAINS*

	BIR-1	BIR-2	BIR-3	Ring Zinc Finger
h-xiap	109 - 312	520 - 723	826 - 1023	1348 - 1485
m-xiap	202 - 405	613 - 816	916 - 1113	1438 - 1575
h-hiap-1	273 - 476	693 - 893	951 - 1154	1824 - 1961
m-hiap-1	251 - 453	670 - 870	928 - 1131	1795 - 1932
h-hiap-2	373 - 576	787 - 987	1042 - 1245	1915 - 2052
m-hiap-2	215 - 418	608 - 808	863 - 1066	1763 - 1876

*Positions indicated correspond to those shown in Figs. 1-4.

TABLE 2

AMINO ACID POSITION OF CONSERVED DOMAINS*

	BIR-1	BIR-2	BIR-3	Ring Zinc Finger
h-XAIP	26 - 93	163 - 230	265 - 330	439 - 484
m-XIAP	26 - 93	163 - 230	264 - 329	438 - 483
h-HIAP1	29 - 96	169 - 235	255 - 322	546 - 591
m-HIAP1	29 - 96	169 - 235	255 - 322	544 - 589
h-HIAP2	46 - 113	184 - 250	269 - 336	560 - 605
m-HIAP2	25 - 92	156 - 222	241 - 308	541 - 578

*Positions indicated correspond to those shown in Figs. 1-4.

Recognition of the mammalian IAP family has provided an emergent pattern of protein structure. Recognition of this pattern allows proteins having a known, homologous sequence but unknown function to be classified as putative inhibitors of apoptosis. A drosophila gene, now termed diap, was classified in this way (for sequence information see Genbank Accession Number M96581 and Fig. 6). The conservation of these proteins across species indicates that the apoptosis signalling pathway has been conserved throughout evolution.

The IAP proteins may be used to inhibit the apoptosis that occurs as part of numerous disease processes or disorders. For example, IAP polypeptides or nucleic acid encoding IAP polypeptides may be administered for the treatment or prevention of apoptosis that occurs as a part of AIDS, neurodegenerative diseases, ischemic injury, toxin-induced liver disease and myelodysplastic syndromes. Nucleic acid encoding the IAP polypeptide may also be provided to inhibit apoptosis.

II. Cloning of IAP Genes

A. xiap

The search for human genes involved in apoptosis resulted in the identification of an X-linked sequence tag site (STS) in the GenBank database, which demonstrated strong homology with the conserved RZF domain of CpIAP and OpIAP, the two baculovirus genes known to inhibit apoptosis (Clem et al., Mol. Cell Biol. 14:5212-5222, 1994; Birnbaum et al., J. Virol. 68:2521-8, 1994). Screening a human fetal brain ZapII cDNA library (Stratagene, La Jolla, CA) with this STS resulted in the identification and cloning of xiap (for X-linked Inhibitor of Apoptosis Protein gene). The human gene has a 1.5 kb coding sequence that includes three BIR domains (Crook et al., J. Virol. 67:2168-74, 1993; Clem et al., Science 254:1388-90, 1991; Birnbaum et al., J. Virol., 68:2521-8, 1994) and a zinc finger. Northern blot analysis with xiap revealed message greater than 7 kb, which is expressed in various tissues, particularly liver and kidney (Fig. 12). The large size of the transcript reflects large 5' and 3' untranslated regions.

B. Human hiap-1 and hiap-2

The hiap-1 and hiap-2 genes were cloned by screening a human liver library (Stratagene Inc., LaJolla, CA) with a probe including the entire xiap coding region at low stringency (the final wash was performed at 40°C with 2X SSC, 10% SDS; Figs. 2 and 3). The hiap-1 and hiap-2 genes were also detected independently using a probe derived from an expressed sequence tag (EST; GenBank Accession No. T96284), which includes a portion of a BIR domain. The EST sequence was originally isolated by the polymerase chain reaction; a cDNA library was used as a template and amplified with EST-specific primers. The DNA ampliderived probe was then used to screen the human liver cDNA library for

full-length hiap coding sequences. A third DNA was subsequently detected that includes the hiap-2 sequence but that appears to lack one exon, presumably due to alternative mRNA splicing (see boxed region in Fig. 3). The expression of hiap-1 and hiap-2 in human tissues as assayed by Northern blot analysis is shown in Figures 8 and 9.

C. m-xiap

Fourteen cDNA and two genomic clones were identified by screening a mouse embryo λgt11 cDNA library (Clontech, Palo Alto, CA) and a mouse FIX II genomic library with a xiap cDNA probe, respectively. A cDNA contig spanning 8.0 kb was constructed using 12 overlapping mouse clones. Sequence analysis revealed a coding sequence of approximately 1.5 kb. The mouse gene, m-xiap, encodes a polypeptide with striking homology to human XIAP at and around the initiation methionine, the stop codon, the three BIR domains, and the RZF domain. As with the human gene, the mouse homologue contains large 5′ and 3′ UTRs, which could produce a transcript as large as 7-8 kb.

Analysis of the sequence and restriction map of m-xiap further delineate the structure and genomic organization of m-xiap. Southern blot analysis and inverse PCR techniques (Groden et al., Cell 66:589-600, 1991) can be employed to map exons and define exon-intron boundaries.

Antisera can be raised against a m-xiap fusion protein that was obtained from, for example, *E. coli* using a bacterial expression system. The resulting antisera can be used along with Northern blot analysis to analyze the spatial and temporal expression of m-xiap in the mouse.

D. m-hiap-1 and m-hiap-2

The murine homologs of hiap-1 and hiap-2 were cloned and sequenced in the same general manner as m-xiap using the human hiap-1 and hiap-2 sequences as probes. Cloning of m-hiap-1 and m-hiap-2 further demonstrate that homologs from different species may be isolated using the techniques provided herein and those generally known to artisans skilled in molecular biology.

III. Identification of Additional IAP Genes

Standard techniques, such as the polymerase chain reaction (PCR) and DNA hybridization, may be used to clone additional human IAP genes and their homologues in other species. Southern blots of human genomic DNA hybridized at low stringency with probes specific for xiap, hiap-1 and hiap-2 reveal bands that correspond to other known human IAP sequences as well as additional bands that do not correspond to known IAP sequences. Thus, additional IAP sequences may be readily identified using low stringency hybridization. Examples of murine and human xiap, hiap-1, and hiap-2 specific primers, which may be used to clone additional genes by RT-PCR, are shown in Table 5.

IV. Characterization of IAP Activity and Intracellular Localization Studies

The ability of putative IAPs to modulate apoptosis can be defined in *in vitro* systems in which alterations of apoptosis can be detected. Mammalian expression constructs carrying IAP cDNAs, which are either full-length or truncated, can be introduced into cell lines such as CHO, NIH 3T3, HL60, Rat-1, or Jurkat cells. In addition, SF21 insect cells may be used, in which case the IAP gene is preferentially expressed using an insect heat shock promotor. Following transfection, apoptosis can be induced by standard methods, which include serum withdrawal, or application of staurosporine, menadione (which induces apoptosis via

free radial formation), or anti-Fas antibodies. As a control, cells are cultured under the same conditions as those induced to undergo apoptosis, but either not transfected, or transfected with a vector that lacks an IAP insert. The ability of each IAP construct to inhibit apoptosis upon expression can be quantified by calculating the survival index of the cells, i.e., the ratio of surviving transfected cells to surviving control cells. These experiments can confirm the presence of apoptosis inhibiting activity and, as discussed below, can also be used to determine the functional region(s) of an IAP. These assays may also be performed in combination with the application of additional compounds in order to identify compounds that modulate apoptosis via IAP expression.

A. Cell Survival following Transfection with Full-length IAP Constructs and Induction of Apoptosis

Specific examples of the results obtained by performing various apoptosis suppression assays are shown in Figs. 14A to 14D. For example, CHO cell survival following transfection with one of six constructs and subsequent serum withdrawal is shown in Fig. 14A. The cells were transfected using Lipofectace™ with 2 μg of one of the following recombinant plasmids: pCDNA36myc-xiap (xiap), pCDNA3-6myc-hiap-1 (hiap-1), pCDNA3-6myc-hiap-2 (hiap-2), pCDNA3-bcl-2 (bcl-2), pCDNA3-HA-smn (smn), and pCDNA3-6myc (6-myc). Oligonucleotide primers were synthesized to allow PCR amplification and cloning of the xiap, hiap-1, and hiap-2 ORFs in pCDNA3 (Invitrogen). Each construct was modified to incorporate a synthetic myc tag encoding six repeats of the peptide sequence MEQKLISEEDL [(SEQ ID NO:__)], thus allowing detection of myc-IAP fusion proteins via monoclonal anti-myc antiserum (Egan et al., Nature 363:45-51, 1993). Triplicate samples of cell lines in 24-well dishes were

washed 5 times with serum free media and maintained in serum free conditions during the course of the experiment. Cells that excluded trypan blue, and that were therefore viable, were counted with a hemocytometer immediately, 24 hours, 48 hours, and 72 hours, after serum withdrawal. Survival was calculated as a percentage of the initial number of viable cells. In this experiment and those presented in Figs. 14B and 14D, the percentage of viable cells shown represents the average of three separate experiments performed in triplicate, +/- average deviation.

The survival of CHO cells following transfection (with each one of the six constructs described above) and exposure to menadione is shown in Fig. 14B. The cells were plated in 24-well dishes, allowed to grow overnight, and then exposed to 20 μM menadione for 1.5 hours (Sigma Chemical Co., St. Louis, MO). Triplicate samples were harvested at the time of exposure to menadione and 24 hours afterward, and survival was assessed by trypan blue exclusion.

The survival of Rat-1 cells following transfection (with each one of the six constructs described above) and exposure to staurosporine is shown in Fig. 14C. Rat-1 cells were transfected and then selected in medium containing 800 μg/ml G418 for two weeks. The cell line was assessed for resistance to staurosporine-induced apoptosis (1 μM) for 5 hours. Viable cells were counted 24 hours after exposure to staurosporine by trypan blue exclusion. The percentage of viable cells shown represents the average of two experiments, ± average deviation.

The Rat-1 cell line was also used to test the resistance of these cells to menadione (Fig. 14D) following transfection with each of the six constructs described above. The cells were exposed to 10 μM

menadione for 1.5 hours, and the number of viable cells was counted 18 hours later.

B. Comparison of Cell Survival Following Transfection with Full-length vs. Partial IAP Constructs

In order to investigate the mechanism whereby human IAPs, including XIAP, HIAP-1, and HIAP-2, afford protection against cell death, expression vectors were constructed that contained either: (1) full-length IAP cDNA (as described above), (2) a portion of an IAP gene that encodes the BIR domains, but not the RZF, or (3) a portion of an IAP gene that encodes the RZF, but not the BIR domains. Human and murine xiap or m-xiap cDNAs were tested by transient or stable expression in HeLa, Jurkat, and CHO cell lines. Following transfection, apoptosis was induced by serum withdrawal, application of menadione, or application of an anti-Fas antibody. Cell death was then assessed, as described above, by trypan blue exclusion. As a control for transfection efficiency, the cells were co-transfected with a β-gal expression construct. Typically, approximately 20% of the cells were successfully transfected.

When CHO cells were transiently transfected, constructs containing full-length xiap or m-xiap cDNAs conferred modest protection against cell death (Fig. 15A). In contrast, the survival of CHO cells transfected with constructs encoding only the BIR domains (i.e., lacking the RZF domain; see Fig. 15A) was markedly enhanced 72 hours after serum deprivation. Furthermore, a large percentage of cells expressing the BIR domains were still viable after 96 hours, at which time no viable cells remained in the control, i.e. non-transfected, cell cultures (see "CHO" in Fig. 15A), and less than 5% of the cells transfected with the vector only, i.e., lacking a cDNA insert, remained viable (see "pcDNA3" in Fig. 15A). Deletion of any of the BIR domains results in the

complete loss of apoptotic suppression, which is reflected by a decrease in the percentage of surviving CHO cells to control levels within 72 hours of serum withdrawal (Fig. 15B; see "xiapΔ1" (which encodes amino acids 89-497 of XIAP (SEQ ID NO.:4)), "xiapΔ2" (which encodes amino acids 246-497 of XIAP (SEQ ID NO.:4)), and "xiapΔ3" (which encodes amino acids 342-497 of XIAP (SEQ ID NO.:4)) at 72 hours).

Stable pools of transfected CHO cells, which were maintained for several months under G418 selection, were induced to undergo apoptosis by exposure to 10 μM menadione for 2 hours. Among the CHO cells tested were those that were stably transfected with: (1) full-length m-xiap cDNA (miap), (2) full-length xiap cDNA (xiap), (3) full-length bcl-2 cDNA (Bcl-2), (4) cDNA encoding the three BIR domains (but not the RZF) of m-xiap (BIR), and (5) cDNA encoding the RZF (but not BIR domains) of m-xiap (RZF). Cells that were non-transfected (CHO) or transfected with the vector only (pcDNA3), served as controls for this experiment. Following exposure to 10 μM menadione, the transfected cells were washed with phosphate buffered saline (PBS) and cultured for an additional 24 hours in menadione-free medium. Cell death was assessed, as described above, by trypan blue exclusion. Less than 10% of the non-transfected or vector-only transfected cells remained viable at the end of the 24 hour survival period. Cells expressing the RZF did not fare significantly better. However, expression of full-length m-xiap, xiap, or bcl-2, and expression of the BIR domains, enhanced cell survival (Fig. 16A). When the concentration of menadione was increased from 10 μM to 20 μM (with all other conditions of the experiment being the same as when 10 μM menadione was applied), the percentage of viable CHO cells that expressed the BIR domain cDNA construct was higher than the percentage of

viable cells that expressed either full-length m-xiap or bcl-2 (Fig. 16B).

C. Analysis of the Subcellular Location of Expressed RZF and BIR Domains

The assays of cell death described above indicate that the RZF may act as a negative regulator of the anti-apoptotic function of IAPs. One way in which the RZF, and possibly other IAP domains, may exert their regulatory influence is by altering the expression of genes, whose products function in the apoptotic pathway.

In order to determine whether the subcellular locations of expressed RZF and BIR domains are consistent with roles as nuclear regulatory factors, COS cells were transiently transfected with the following four constructs, and the expressed polypeptide was localized by immunofluorescent microscopy: (1) pcDNA3-6myc-xiap, which encodes all 497 amino acids of SEQ ID NO:4, (2) pcDNA3-6myc-m-xiap, which encodes all 497 amino acids of mouse xiap (SEQ ID NO:10), (3) pcDNA3-6myc-mxiap-BIR, which encodes amino acids 1 to 341 of m-xiap (SEQ ID NO:10), and (4) pcDNA3-6myc-mxiap-RZF, which encodes amino acids 342-497 of m-xiap (SEQ ID NO:10). The cells were grown on multi-well tissue culture slides for 12 hours, and then fixed and permeabilized with methanol. The constructs used (here and in the cell death assays) were tagged with a human Myc epitope tag at the N-terminus. Therefore, a monoclonal anti-Myc antibody and a secondary goat anti-mouse antibody, which was conjugated to FITC, could be used to localize the expressed products in transiently transfected COS cells. Full-length XIAP and MIAP were located in the cytoplasm, with accentuated expression in the peri-nuclear zone. The same pattern of localization was observed when the cells expressed a construct encoding the RZF domain (but not the BIR domains). However, cells expressing the BIR domains (without the RZF) exhibited, primarily, nuclear

staining. The protein expressed by the BIR domain construct appeared to be in various stages of transfer to the nucleus.

These observations are consistent with the fact that, as described below, XIAP is cleaved within T cells that are treated with anti-Fas antibodies (which are potent inducers of apoptosis), and its N-terminal domain is translocated to the nucleus.

D. Examples of Additional Apoptosis Assays

Specific examples of apoptosis assays are also provided in the following references. Assays for apoptosis in lymphocytes are disclosed by: Li et al., "Induction of apoptosis in uninfected lymphocytes by HIV-1 Tat protein", Science 268:429-431, 1995; Gibellini et al., "Tat-expressing Jurkat cells show an increased resistance to different apoptotic stimuli, including acute human immunodeficiency virus-type 1 (HIV-1) infection", Br. J. Haematol. 89:24-33, 1995; Martin et al., "HIV-1 infection of human $CD4^+$ T cells *in vitro*. Differential induction of apoptosis in these cells." J. Immunol. 152:330-42, 1994; Terai et al., "Apoptosis as a mechanism of cell death in cultured T lymphoblasts acutely infected with HIV-1", J. Clin Invest. 87:1710-5, 1991; Dhein et al., "Autocrine T-cell suicide mediated by APO-1/(Fas/CD95)11, Nature 373:438-441, 1995; Katsikis et al., "Fas antigen stimulation induces marked apoptosis of T lymphocytes in human immunodeficiency virus-infected individuals", J. Exp. Med. 1815:2029-2036, 1995; Westendorp et al., Sensitization of T cells to CD95-mediated apoptosis by HIV-1 Tat and gp120", Nature 375:497, 1995; DeRossi et al., Virology 198:234-44, 1994.

Assays for apoptosis in fibroblasts are disclosed by: Vossbeck et al., "Direct transforming activity of TGF-beta on rat fibroblasts", Int. J. Cancer 61:92-97, 1995; Goruppi et al., "Dissection of c-myc

domains involved in S phase induction of NIH3T3 fibroblasts", Oncogene 9:1537-44, 1994; Fernandez et al., "Differential sensitivity of normal and Ha-ras transformed C3H mouse embryo fibroblasts to tumor necrosis factor: induction of bcl-2, c-myc, and manganese superoxide dismutase in resistant cells", Oncogene 9:2009-17, 1994; Harrington et al., "c-Myc-induced apoptosis in fibroblasts is inhibited by specific cytokines", EMBO J., 13:3286-3295, 1994; Itoh et al., "A novel protein domain required for apoptosis. Mutational analysis of human Fas antigen", J. Biol. Chem. 268:10932-7, 1993.

Assays for apoptosis in neuronal cells are disclosed by: Melino et al., "Tissue transglutaminase and apoptosis: sense and antisense transfection studies with human neuroblastoma cells", Mol. Cell Biol. 14:6584-6596, 1994; Rosenbaum et al., "Evidence for hypoxia-induced, programmed cell death of cultured neurons", Ann. Neurol. 36:864-870, 1994; Sato et al., "Neuronal differentiation of PC12 cells as a result of prevention of cell death by bcl-2", J. Neurobiol 25:1227-1234, 1994; Ferrari et al., "N-acetylcysteine D- and L-stereoisomers prevents apoptotic death of neuronal cells", J. Neurosci. 1516:2857-2866, 1995; Talley et al., "Tumor necrosis factor alpha-induced apoptosis in human neuronal cells: protection by the antioxidant N-acetylcysteine and the genes bcl-2 and crma", Mol. Cell Biol. 1585:2359-2366, 1995; Talley et al., "Tumor Necrosis Factor Alpha-Induced Apoptosis in Human Neuronal Cells: Protection by the Antioxidant NAcetylcysteine and the Genes bcl-2 and crma", Mol. Cell. Biol. 15:2359-2366, 1995; Walkinshaw et al., "Induction of apoptosis in catecholaminergic PC12 cells by L-DOPA. Implications for the treatment of Parkinson's disease.", J. Clin. Invest. 95:2458-2464, 1995.

Assays for apoptosis in insect cells are disclosed by: Clem et al., "Prevention of apoptosis by a baculovirus gene during infection of insect cells", Science 254:1388-90, 1991; Crook et al., "An apoptosis-inhibiting baculovirus gene with a zinc finger-like motif", J. Virol. 67:2168-74, 1993; Rabizadeh et al., "Expression of the baculovirus p35 gene inhibits mammalian neural cell death", J. Neurochem. 61:2318-21, 1993; Birnbaum et al., "An apoptosis inhibiting gene from a nuclear polyhedrosis virus encoding a polypeptide with Cys/His sequence motifs", J. Virol. 68:2521-8, 1994; Clem et al., "Control of programmed cell death by the baculovirus genes p35 and IAP", Mol. Cell. Biol. 14:5212-5222, 1994.

V. Construction of a Transgenic Animal

Characterization of IAP genes provides information that is necessary for an IAP knockout animal model to be developed by homologous recombination. Preferably, the model is a mammalian animal, most preferably a mouse. Similarily, an animal model of IAP overproduction may be generated by integrating one or more IAP sequences into the genome, according to standard transgenic techniques.

A replacement-type targeting vector, which would be used to create a knockout model, can be constructed using an isogenic genomic clone, for example, from a mouse strain such as 129/Sv (Stratagene Inc., LaJolla, CA). The targeting vector will be introduced into a suitably-derived line of embryonic stem (ES) cells by electroporation to generate ES cell lines that carry a profoundly truncated form of an IAP. To generate chimeric founder mice, the targeted cell lines will be injected into a mouse blastula stage embryo. Heterozygous offspring will be interbred to homozygosity. Knockout mice would provide the means, *in vivo*, to screen

for therapeutic compounds that modulate apoptosis via an IAP-dependent pathway.

VI. IAP Protein Expression

IAP genes may be expressed in both prokaryotic and eukaryotic cell types. If an IAP modulates apoptosis by exacerbating it, it may be desirable to express that protein under control of an inducible promotor.

In general, IAPs according to the invention may be produced by transforming a suitable host cell with all or part of an IAP-encoding cDNA fragment that has been placed into a suitable expression vector.

Those skilled in the art of molecular biology will understand that a wide variety of expression systems may be used to produce the recombinant protein. The precise host cell used is not critical to the invention. The IAP protein may be produced in a prokaryotic host (e.g., *E. coli*) or in a eukaryotic host (e.g., *S. cerevisiae*, insect cells such as Sf21 cells, or mammalian cells such as COS-1, NIH 3T3, or HeLa cells). These cells are publically available, for example, from the American Type Culture Collection, Rockville, MD; see also Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY, 1994). The method of transduction and the choice of expression vehicle will depend on the host system selected. Transformation and transfection methods are described, e.g., in Ausubel et al. (*supra*), and expression vehicles may be chosen from those provided, e.g. in Cloning Vectors: A Laboratory Manual (P.H. Pouwels et al., 1985, Supp. 1987).

A preferred expression system is the baculovirus system using, for example, the vector pBacPAK9, which is available from Clontech (Palo Alto, CA). If desired, this system may be used in conjunction with other protein expression techniques, for example, the myc tag approach

described by Evan et al. (Mol. Cell Biol. 5:3610-3616, 1985).

Alternatively, an IAP may be produced by a stably-transfected mammalian cell line. A number of vectors suitable for stable transfection of mammalian cells are available to the public, e.g., see Pouwels et al. (*supra*), as are methods for constructing such cell lines (see e.g., Ausubel et al. (*supra*). In one example, cDNA encoding an IAP is cloned into an expression vector that includes the dihydrofolate reductase (DHFR) gene. Integration of the plasmid and, therefore, integration of the IAP-encoding gene into the host cell chromosome is selected for by inclusion of 0.01-300 μM methotrexate in the cell culture medium (as described, Ausubel et al., *supra*). This dominant selection can be accomplished in most cell types. Recombinant protein expression can be increased by DHFR-mediated amplification of the transfected gene.

Methods for selecting cell lines bearing gene amplifications are described in Ausubel et al. (*supra*). These methods generally involve extended culture in medium containing gradually increasing levels of methotrexate. The most commonly used DHFR-containing expression vectors are pCVSEII-DHFR and pAdD26SV(A) (described in Ausubel et al., *supra*). The host cells described above or, preferably, a DHFR-deficient CHO cell line (e.g., CHO DHFR$^-$ cells, ATCC Accession No. CRL 9096) are among those most preferred for DHFR selection of a stably-transfected cell line or DHFR-mediated gene amplification.

Once the recombinant protein is expressed, it is isolated by, for example, affinity chromatography. In one example, an anti-IAP antibody, which may be produced by the methods described herein, can be attached to a column and used to isolate the IAP protein. Lysis and

fractionation of IAP-harboring cells prior to affinity chromatography may be performed by standard methods (see e.g., Ausubel et al., *supra*). Once isolated, the recombinant protein can, if desired, be purified further by e.g., by high performance liquid chromatography (HPLC; e.g., see Fisher, Laboratory Techniques In Biochemistry And Molecular Biology, Work and Burdon, Eds., Elsevier, 1980).

Polypeptides of the invention, particularly short IAP fragments, can also be produced by chemical synthesis (e.g., by the methods described in Solid Phase Peptide Synthesis, 2nd ed., 1984 The Pierce Chemical Co., Rockford, IL). These general techniques of polypeptide expression and purification can also be used to produce and isolate useful IAP fragments or analogs, asdescribed herein.

VII. Anti-IAP Antibodies

In order to generate IAP-specific antibodies, an IAP coding sequence (i.e., amino acids 180-276) can be expressed as a C-terminal fusion with glutathione S-transferase (GST; Smith et al., Gene 67:31-40, 1988). The fusion protein can be purified on glutathione-Sepharose beads, eluted with glutathione, and cleaved with thrombin (at the engineered cleavage site), and purified to the degree required to successfully immunize rabbits. Primary immunizations can be carried out with Freund's complete adjuvant and subsequent immunizations performed with Freund's incomplete adjuvant. Antibody titres are monitored by Western blot and immunoprecipitation analyses using the thrombin-cleaved IAP fragment of the GST-IAP fusion protein. Immune sera are affinity purified using CNBr-Sepharose-coupled IAP protein. Antiserum specificity is determined using a panel of unrelated GST proteins (including GSTp53, Rb,

HPV-16 E6, and E6-AP) and GST-trypsin (which was generated by PCR using known sequences).

As an alternate or adjunct immunogen to GST fusion proteins, peptides corresponding to relatively unique hydrophilic regions of IAP may be generated and coupled to keyhole limpet hemocyanin (KLH) through an introduced C-terminal lysine. Antiserum to each of these peptides is similarly affinity purified on peptides conjugated to BSA, and specificity is tested by ELISA and Western blotting using peptide conjugates, and by Western blotting and immunoprecipitation using IAP expressed as a GST fusion protein.

Alternatively, monoclonal antibodies may be prepared using the IAP proteins described above and standard hybridoma technology (see, e.g., Kohler et al., Nature 256:495, 1975; Kohler et al., Eur. J. Immunol. 6:511, 1976;
Kohler et al., Eur. J. Immunol. 6:292, 1976; Hammerling et al., In Monoclonal Antibodies and T Cell Hybridomas, Elsevier, New York, NY, 1981; Ausubel et al., *supra*). Once produced, monoclonal antibodies are also tested for specific IAP recognition by Western blot or immunoprecipitation analysis (by the methods described in Ausubel et al., *supra*).

Antibodies that specifically recognize IAPs or fragments of IAPs, such as those described herein containing one or more BIR domains (but not a ring zinc finger domain), or that contain a ring zinc finger domain (but not a BIR domain) are considered useful in the invention. They may, for example, be used in an immunoassay to monitor IAP expression levels or to determine the subcellular location of an IAP or IAP fragment produced by a mammal. Antibodies that inhibit the 26 kDa IAP cleavage product described herein (which contains at least one BIR domain) may be especially

useful in inducing apoptosis in cells undergoing undesirable proliferation.

Preferably, antibodies of the invention are produced using IAP sequence that does not reside within highly conserved regions, and that appears likely to be antigenic, as analyzed by criteria such as those provided by the Peptide structure program (Genetics Computer Group Sequence Analysis Package, Program Manual for the GCG Package, Version 7, 1991) using the algorithm of Jameson and Wolf (CABIOS 4:181, 1988). Specifically, these regions, which are found between BIR1 and BIR2 of all IAPs, are: from amino acid 99 to amino acid 170 of hiap-1, from amino acid 123 to amino acid 184 of hiap-2, and from amino acid 116 to amino acid 133 of either xiap or m-xiap. These fragments can be generated by standard techniques, e.g. by the PCR, and cloned into the pGEX expression vector (Ausubel et al., *supra*). Fusion proteins are expressed in *E. coli* and purified using a glutathione agarose affinity matrix as described in Ausubel et al. (*supra*). In order to minimize the potential for obtaining antisera that is non-specific, or exhibits low-affinity binding to IAP, two or three fusions are generated for each protein, and each fusion is injected into at least two rabbits. Antisera are raised by injections in series, preferably including at least three booster injections.

VIII. Identification of Molecules that Modulate IAP Protein Expression

Isolation of IAP cDNAs also facilitates the identification of molecules that increase or decrease IAP expression. In one approach, candidate molecules are added, in varying concentration, to the culture medium of cells expressing IAP mRNA. IAP expression is then measured, for example, by Northern blot analysis (Ausubel et al., *supra*) using an IAP cDNA, or cDNA fragment, as a hybridization probe (see also Table 5). The level of IAP

expression in the presence of the candidate molecule is compared to the level of IAP expression in the absence of the candidate molecule, all other factors (e.g. cell type and culture conditions) being equal.

The effect of candidate molecules on IAP-mediated apoptosis may, instead, be measured at the level of translation by using the general approach described above with standard protein detection techniques, such as Western blotting or immunoprecipitation with an IAP-specific antibody (for example, the IAP antibody described herein).

Compounds that modulate the level of IAP may be purified, or substantially purified, or may be one component of a mixture of compounds such as an extract or supernatant obtained from cells (Ausubel et al., *supra*). In an assay of a mixture of compounds, IAP expression is tested against progressively smaller subsets of the compound pool (e.g., produced by standard purification techniques such as HPLC or FPLC) until a single compound or minimal number of effective compounds is demonstrated to modulate IAP expression.

Compounds may also be screened for their ability to modulate IAP apoptosis inhibiting activity. In this approach, the degree of apoptosis in the presence of a candidate compound is compared to the degree of apoptosis in its absence, under equivalent conditions. Again, the screen may begin with a pool of candidate compounds, from which one or more useful modulator compounds are isolated in a step-wise fashion. Apoptosis activity may be measured by any standard assay, for example, those described herein.

Another method for detecting compounds that modulate the activity of IAPs is to screen for compounds that interact physically with a given IAP polypeptide. These compounds may be detected by adapting interaction

trap expression systems known in the art. These systems detect protein interactions using a transcriptional activation assay and are generally described by Gyuris et al. (Cell 75:791-803, 1993) and Field et al., Nature 340:245-246, 1989), and are commercially available from Clontech (Palo Alto, CA). In addition, PCT Publication WO 95/28497 describes an interaction trap assay in which proteins involved in apoptosis, by virtue of their interaction with Bcl-2, are detected. A similar method may be used to identify proteins and other compounds that interact with IAPs.

Compounds or molecules that function as modulators of IAP-mediated cell death may include peptide and non-peptide molecules such as those present in cell extracts, mammalian serum, or growth medium in which mammalian cells have been cultured.

A molecule that promotes an increase in IAP expression or IAP activity is considered particularly useful in the invention; such a molecule may be used, for example, as a therapeutic to increase cellular levels of IAP and thereby exploit the ability of IAP polypeptides to inhibit apoptosis.

A molecule that decreases IAP activity (e.g., by decreasing IAP gene expression or polypeptide activity) may be used to decrease cellular proliferation. This would be advantageous in the treatment of neoplasms (see Table 3, below), or other cell proliferative diseases.

TABLE 3

NORTHERN BLOT IAP RNA LEVELS IN CANCER CELLS*

	xiap	hiap1	hiap2
Promyelocytic Leukemia HL-60	+	+	+
Hela S-3	+	+	+
Chronic Myelogenous Leukemia K-562	+++	+	+++
Lymphoblastic Leukemia MOLT-4	+++	+	+
Burkitt's Lymphoma Raji	+	+(x10)	+
Colorectal Adenocarcinoma SW-480	+++	+++	+++
Lung Carcinoma A-549	+	+	+
Melanoma G-361	+++	+	+

*Levels are indicated by a (+) and are the approximate increase in RNA levels relative to Northern blots of RNA from non-cancerous control cell lines. A single plus indicates an estimated increase of at least 1-fold

Molecules that are found, by the methods described above, to effectively modulate IAP gene expression or polypeptide activity may be tested further in animal models. If they continue to function successfully in an *in vivo* setting, they may be used as therapeutics to either inhibit or enhance apoptosis, as appropriate.

IX. IAP Therapy

The level of IAP gene expression correlates with the level of apoptosis. Thus, IAP genes also find use in anti-apoptosis gene therapy. In particular, a functional IAP gene may be used to sustain neuronal cells that undergo apoptosis in the course of a neurodegenerative disease, lymphocytes (i.e., T cells and B cells), or cells that have been injured by ischemia.

Retroviral vectors, adenoviral vectors, adeno-associated viral vectors, or other viral vectors with the appropriate tropism for cells likely to be involved in

apoptosis (for example, epithelial cells) may be used as a gene transfer delivery system for a therapeutic IAP gene construct. Numerous vectors useful for this purpose are generally known (Miller, Human Gene Therapy 15-14, 1990; Friedman, Science 244:1275-1281, 1989; Eglitis and Anderson, BioTechniques 6:608-614, 1988; Tolstoshev and Anderson, current opinion in Biotechnology 1:55-61, 1990; Sharp, The Lancet 337:1277-1278, 1991; Cornetta et al., Nucleic Acid Research and Molecular Biology 36:311-322, 1987; Anderson, Science 226:401-409, 1984; Moen, Blood Cells 17:407-416, 1991; Miller et al., Biotechniques 7:980-990, 1989; Le Gal La Salle et al., Science 259:988-990, 1993; and Johnson, Chest 107:77S-83S, 1995). Retroviral vectors are particularly well developed and have been used in clinical settings (Rosenberg et al., N. Engl. J. Med 323:370, 1990; Anderson et al., U.S. Patent No. 5,399,346). Non-viral approaches may also be employed for the introduction of therapeutic DNA into cells otherwise predicted to undergo apoptosis. For example, IAP may be introduced into a neuron or a T cell by lipofection (Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413, 1987; Ono et al., Neurosci. Lett. 117:259, 1990; Brigham et al., Am. J. Med. Sci. 298:278, 1989; Staubinger et al., Meth. Enz. 101:512, 1983), asialorosonucoid-polylysine conjugation (Wu et al., J. Biol. Chem. 263:14621, 1988; Wu et al., J. Biol. Chem. 264:16985, 1989); or, less preferably, microinjection under surgical conditions (Wolff et al., Science 247:1465, 1990).

For any of the methods of application described above, the therapeutic IAP DNA construct is preferably applied to the site of the predicted apoptosis event (for example, by injection). However, it may also be applied to tissue in the vicinity of the predicted apoptosis

event or to a blood vessel supplying the cells predicted to undergo apoptosis.

In the constructs described, IAP cDNA expression can be directed from any suitable promoter (e.g., the human cytomegalovirus (CMV), simian virus 40 (SV40), or metallothionein promoters), and regulated by any appropriate mammalian regulatory element. For example, if desired, enhancers known to preferentially direct gene expression in neural cells, T cells, or B cells may be used to direct IAP expression. The enhancers used could include, without limitation, those that are characterized as tissue- or cell-specific in their expression. Alternatively, if an IAP genomic clone is used as a therapeutic construct (for example, following its isolation by hybridization with the IAP cDNA described above), regulation may be mediated by the cognate regulatory sequences or, if desired, by regulatory sequences derived from a heterologous source, including any of the promoters or regulatory elements described above.

Less preferably, IAP gene therapy is accomplished by direct administration of the IAP mRNA or antisense IAP mRNA to a cell that is expected to undergo apoptosis. The mRNA may be produced and isolated by any standard technique, but is most readily produced by *in vitro* transcription using an IAP cDNA under the control of a high efficiency promoter (e.g., the T7 promoter). Administration of IAP mRNA to malignant cells can be carried out by any of the methods for direct nucleic acid administration described above.

Ideally, the production of IAP protein by any gene therapy approach will result in cellular levels of IAP that are at least equivalent to the normal, cellular level of IAP in an unaffected cell. Treatment by any

IAP-mediated gene therapy approach may be combined with more traditional therapies.

Another therapeutic approach within the invention involves administration of recombinant IAP protein, either directly to the site of a predicted apoptosis event (for example, by injection) or systemically (for example, by any conventional recombinant protein administration technique). The dosage of IAP depends on a number of factors, including the size and health of the individual patient, but, generally, between 0.1 mg and 100 mg inclusive are administered per day to an adult in any pharmaceutically-acceptable formulation.

X. Administration of IAP Polypeptides, IAP Genes, or Modulators of IAP Synthesis or Function

An IAP protein, gene, or modulator may be administered within a pharmaceutically-acceptable diluent, carrier, or excipient, in unit dosage form. Conventional pharmaceutical practice may be employed to provide suitable formulations or compositions to administer IAP to patients suffering from a disease that is caused by excessive apoptosis. Administration may begin before the patient is symptomatic. Any appropriate route of administration may be employed, for example, administration may be parenteral, intravenous, intraarterial, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, aerosol, or oral administration. Therapeutic formulations may be in the form of liquid solutions or suspensions; for oral administration, formulations may be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols.

Methods well known in the art for making formulations are found, for example, in "Remington's

Pharmaceutical Sciences." Formulations for parenteral administration may, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated napthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for IAP modulatory compounds include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel.

If desired, treatment with an IAP protein, gene, or modulatory compound may be combined with more traditional therapies for the disease such as surgery, steroid therapy, or chemotherapy for autoimmune disease; antiviral therapy for AIDS; and tissue plasminogen activator (TPA) for ischemic injury.

XI. Detection of Conditions Involving Altered Apoptosis

IAP polypeptides and nucleic acid sequences find diagnostic use in the detection or monitoring of conditions involving aberrant levels of apoptosis. For example, decrease expression of IAP may be correlated with enhanced apoptosis in humans (see XII, below). Accordingly, a decrease or increase in the level of IAP production may provide an indication of a deleterious condition. Levels of IAP expression may be assayed by any standard technique. For example, IAP expression in a biological sample (e.g., a biopsy) may be monitored by standard Northern blot analysis or may be aided by PCR

(see, e.g., Ausubel et al., *supra*; PCR Technology: Principles and Applications for DNA Amplification, H.A. Ehrlich, Ed. Stockton Press, NY; Yap et al. Nucl. Acids. Res. 19:4294, 1991).

Alternatively, a biological sample obtained from a patient may be analyzed for one or more mutations in the IAP sequences using a mismatch detection approach. Generally, these techniques involve PCR amplification of nucleic acid from the patient sample, followed by identification of the mutation (i.e., mismatch) by either altered hybridization, aberrant electrophoretic gel migration, binding or cleavage mediated by mismatch binding proteins, or direct nucleic acid sequencing. Any of these techniques may be used to facilitate mutant IAP detection, and each is well known in the art; examples of particular techniques are described, without limitation, in Orita et al., Proc. Natl. Acad. Sci. USA 86:2766-2770, 1989; Sheffield et al., Proc. Natl. Acad. Sci. USA 86:232-236, 1989).

In yet another approach, immunoassays are used to detect or monitor IAP protein in a biological sample. IAPspecific polyclonal or monoclonal antibodies (produced as described above) may be used in any standard immunoassay format (e.g., ELISA, Western blot, or RIA) to measure IAP polypeptide levels. These levels would be compared to wild-type IAP levels, with a decrease in IAP production indicating a condition involving increased apoptosis. Examples of immunoassays are described, e.g., in Ausubel et al., *supra*. Immunohistochemical techniques may also be utilized for IAP detection. For example, a tissue sample may be obtained from a patient, sectioned, and stained for the presence of IAP using an anti-IAP antibody and any standard detection system (e.g., one which includes a secondary antibody conjugated to horseradish peroxidase). General guidance regarding such

techniques can be found in, e.g., Bancroft and Stevens (Theory and Practice of Histological Techniques, Churchill Livingstone, 1982) and Ausubel et al. (*supra*).

In one preferred example, a combined diagnostic method may be employed that begins with an evaluation of IAP protein production (for example, by immunological techniques or the protein truncation test (Hogerrorst et al., Nature Genetics 10:208-212, 1995) and also includes a nucleic acid-based detection technique designed to identify more subtle IAP mutations (for example, point mutations). As described above, a number of mismatch detection assays are available to those skilled in the art, and any preferred technique may be used. Mutations in IAP may be detected that either result in loss of IAP expression or loss of IAP biological activity. In a variation of this combined diagnostic method, IAP biological activity is measured as protease activity using any appropriate protease assay system (for example, those described above).

Mismatch detection assays also provide an opportunity to diagnose an IAP-mediated predisposition to diseases caused by inappropriate apoptosis. For example, a patient heterozygous for an IAP mutation may show no clinical symptoms and yet possess a higher than normal probability of developing one or more types of neurodegenerative, myelodysplastic or ischemic diseases. Given this diagnosis, a patient may take precautions to minimize their exposure to adverse environmental factors (for example, UV exposure or chemical mutagens) and to carefully monitor their medical condition (for example, through frequent physical examinations). This type of IAP diagnostic approach may also be used to detect IAP mutations in prenatal screens. The IAP diagnostic assays described above may be carried out using any biological sample (for example, any biopsy sample or bodily fluid or

tissue) in which IAP is normally expressed. Identification of a mutant IAP gene may also be assayed using these sources for test samples.

Alternatively, a IAP mutation, particularly as part of a diagnosis for predisposition to IAP-associated degenerative disease, may be tested using a DNA sample from any cell, for example, by mismatch detection techniques. Preferably, the DNA sample is subjected to PCR amplification prior to analysis.

In order to demonstrate the utility of IAP gene sequences as diagnostics and prognostics for cancer, a Human Cancer Cell Line Multiple Tissue Northern Blot (Clontech, Palo Alto, CA; #7757-1) was probed. This Northern blot contained approximately 2 μg of poly A^+ RNA per lane from eight different human cell lines: (1) promyelocytic leukemia HL-60, (2) HeLa cell S3, (3) chronic myelogenous leukemia K-562, (4) lymphoblastic leukemia MOLT-4, (5) Burkitt's lymphoma Raji, (6) colorectal adenocarcinoma SW480, (7) lung carcinoma A549, and (8) melanoma G361. As a control, a Human Multiple Tissue Northern Blot (Clontech, Palo Alto, CA; #7759-1) was probed. This Northern blot contained approximately 2 μg of poly A^+ RNA from eight different human tissues: (1) spleen, (2) thymus, (3) prostate, (4) testis, (5) ovary, (6) small intestine, (7) colon, and (8) peripheral blood leukocytes.

The Northern blots were hybridized sequentially with: (1) a 1.6 kb probe to the xiap coding region, (2) a 375 bp hiap-2 specific probe corresponding to the 3′ untranslated region, (3) a 1.3 kb probe to the coding region of hiap-1, which cross-reacts with hiap-2, (4) a 1.0 kb probe derived from the coding region of bcl-2, and (5) a probe to β-actin, which was provided by the manufacturer. Hybridization was carried out at 50°C overnight, according to the manufacturer's suggestion.

The blot was washed twice with 2X SSC, 0.1% SDS at room temperature for 15 minutes and then with 2X SSC, 0.1% SDS at 50°C.

All cancer lines tested showed increased IAP expression relative to samples from non-cancerous control tissues (Table 3). Expression of xiap was particularly high in HeLa (S-3), chronic myelogenous leukemia (K-562), colorectal adenocarcinoma (SW-480), and melanoma (G-361) lines. Expression of hiap-1 was extremely high in Burkitt's lymphoma, and was also elevated in colorectal adenocarcinoma. Expression of hiap-2 was particularly high in chronic myelogenous leukemia (K-562) and colorectal adenocarcinoma (SW-480). Expression of Bcl-2 was upregulated only in HL-60 leukemia cells.

These observations suggest that upregulation of the anti-apoptotic IAP genes may be a widespread phenomenon, perhaps occurring much more frequently than upregulation of Bcl-2. Furthermore, upregulation may be necessary for the establishment or maintenance of the transformed state of cancerous cells.

In order to pursue the observation described above, i.e., that hiap-1 is overexpressed in the Raji Burkitt's lymphoma cell line, RT-PCR analysis was performed in multiple Burkitt's lymphoma cell lines. Total RNA was extracted from cells of the Raji, Ramos, EB-3, and Jiyoye cell lines, and as a positive control, from normal placental tissue. The RNA was reverse transcribed, and amplified by PCR with the following set of oligonucleotide primers:

5'-AGTGCGGGTTTTTATTATGTG-3' (SEQ ID NO:__) and 5'-AGATGACCACAAGGAATAAACACTA-3' (SEQ ID NO:__), which selectively amplify a hiap-1 cDNA fragment. RT-PCR was conducted using a PerkinElmer 480 Thermocycler to carry out 35 cycles of the following program: 94°C for 1 minute, 50°C for 1.5 minutes, and 72°C for a minute. The

PCR reaction product was electrophoresed on an agarose gel and stained with Ethidium bromide. Amplified cDNA fragments of the appropriate size were clearly visible in all lanes containing Burkitt's lymphoma samples, but absent in the lanes containing the normal placental tissue sample, and absent in lanes containing negative control samples, where template DNA was omitted from the reaction (Fig. 17).

XII. Accumulation of a 26 kDa Cleavage Protein in Astrocytoma Cells

A. Identification of a 26 kDa Cleavage Protein

A total protein extract was prepared from Jurkat and astrocytoma cells by sonicating them (X3 for 15 seconds at 4°C) in 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM PMSF, 1 μg/ml aprotinin, and 5 mM benzamidine. Following sonication, the samples were centrifuged (14,000 RPM in a microfuge) for five minutes. Twenty μg of protein was loaded per well on a 10% SDS-polyacrylamide gel, electrophoresed, and electroblotted by standard methods to PVDF membranes. Western blot analysis, performed as described previously, revealed that the astrocytoma cell line (CCF-STTG1) abundantly expressed an anti-xiap reactive band of approximately 26 kDa, despite the lack of an apoptotic trigger event (Fig. 18). In fact, this cell line has been previously characterized as being particularly resistant to standard apoptotic triggers.

A 26 kDa xiap-reactive band was also observed under the following experimental conditions. Jurkat cells (a transformed human T cell line) were induced to undergo apoptosis by exposure to an anti-Fas antibody (1 μg/ml). Identical cultures of Jurkat cells were exposed either to: (1) anti-Fas antibody and cycloheximide (20 μg/ml), (2) tumor necrosis factor alpha (TNF-α, at 1,000 U/ml), or (3) TNF-α and cycloheximide (20 μg/ml). All cells were harvested 6 hours after treatment began. In

addition, as a negative control, anti-Fas antibody was added to an extract after the cells were harvested. The cells were harvested in SDS sample buffer, electrophoresed on a 12.5% SDS polyacrylamide gel, and electroblotted onto PVDF membranes using standard methods. The membranes were immunostained with a rabbit polyclonal anti-XIAP antibody at 1:1000 for 1 hour at room temperature. Following four 15 minute washes, a goat anti-rabbit antibody conjugated to horse-radish peroxidase was applied at room temperature for 1 hour. Unbound secondary antibody was washed away, and chemiluminescent detection of XIAP protein was performed. The Western blot revealed the presence of the full-length, 55 kDa XIAP protein, both in untreated and treated cells. In addition, a novel, approximately 26 kDa xiap-reactive band was also observed in apoptotic cell extracts, but not in the control, untreated cell extracts (Fig. 19).

Cleavage of XIAP occurs in a variety of cell types, including other cancer cell lines such as HeLa. The expression of the 26 kDa XIAP cleavage product was demonstrated in HeLa cells as follows. HeLa cells were treated with either: (1) cyclohexamide (20 μg/ml), (2) anti-Fas antibody (1 μg/ml), (3) anti-Fas antibody (1 μg/ml) and cyclohexamide (20 μg/ml), (4) TNFα (1,000 U/ml), or (5) TNFα (1,000 U/ml) and cyclohexamide (20 μg/ml). All cells were harvested 18 hours after treatment began. As above, anti-Fas antibody was added to an extract after the cells were harvested. HeLa cells were harvested, and the Western blot was probed under the same conditions as used to visualize xiap-reactive bands from Jurkat cell samples. A 26 kDa XIAP band was again seen in the apoptotic cell preparations (Fig. 20). Furthermore, the degree of XIAP cleavage correlated positively with the extent of apoptosis. Treatment of

HeLa cells with cycloheximide or TNFα alone caused only minor apoptosis, and little cleavage product was observed. If the cells were treated with the anti-Fas antibody, a greater amount of cleavage product was apparent. These data indicate that XIAP is cleaved in more than one cell type and in response to more than one type of apoptotic trigger.

B. Time Course of Expression

The time course over which the 26 kDa cleavage product accumulates was examined by treating HeLa and Jurkat cells with anti-Fas antibody (1 μg/ml) and harvesting them either immediately, or 1, 2, 3, 5, 10, or 22 hours after treament. Protein extracts were prepared and Western blot analysis was performed as described above. Both types of cells accumulated increasing quantities of the 26 kDa cleavage product over the time course examined (Figs. 21A and 21B).

C. Subcellular Localization of the 26 kDa XIAP Cleavage Product

In order to determine the subcellular location of the 26 kDa cleavage product, Jurkat cells were induced to undergo apoptosis by exposure to anti-Fas antibody (1 μg/ml) and were then harvested either immediately, 3 hours, or 7 hours later. Total protein extracts were prepared, as described above, from cells harvested at each time point. In order to prepare nuclear and cytoplasmic cell extracts, apoptotic Jurkat cells were washed with isotonic Tris buffered saline (pH 7.0) and lysed by freezing and thawing five times in cell extraction buffer (50 mM PIPES, 50 mM KCl, 5 mM EGTA, 2 mM $MgCl_2$, 1 mM DTT, and 20 μM cytochalasin B). Nuclei were pelleted by centrifugation and resuspended in isotonic Tris (pH 7.0) and frozen at -80°C. The cytoplasmic fraction of the extract was processed further by centrifugation at 60,000 RPM in a TA 100.3 rotor for 30 minutes. Supernatants were removed and frozen at -

80°C. Samples of both nuclear and cytoplasmic fractions were loaded on a 12.5% SDS-polyacrylamide gel, and electroblotted onto PVDF membranes. Western blot analysis was then performed using either an anti-CPP32 antibody (Transduction Laboratories Lexington, KY; Fig. 22A) or the rabbit anti-XIAP antibody described above (Fig. 22B).

The anti-CPP32 antibody, which recognizes the CPP32 protease (also known as YAMA or Apopain) partitioned almost exclusively in the cytoplasmic fraction. The 55 kDa XIAP protein localized exclusively in the cytoplasm of apoptotic cells, in agreement with the studies presented above, where XIAP protein in normal, healthy COS cells was seen to localize, by immunofluoresence microscopy, to the cytoplasm. In contrast, the 26 kDa cleavage product localized exclusively to the nuclear fraction of apoptotic Jurkat cells. Taken together, these observations suggest that the anti-apoptotic component of XIAP could be the 26 kDa cleavage product, which exerts its influence within the nucleus.

D. In vitro Cleavage of XIAP protein and Characterization of the Cleavage Product

For this series of experiments, XIAP protein was labeled with ^{35}S using the plasmid pcDNA3-6myc-XIAP, T7 RNA polymerase, and a coupled transcription/translation kit (Promega) according to the manufacturer's instructions. Radioactively labeled XIAP protein was separated from unincorporated methionine by column chromatography using Sephadex G-50™. In addition, extracts of apoptotic Jurkat cells were prepared following treatment with anti-Fas antibody (1 μg/ml) for three hours. To prepare the extracts, the cells were lysed in Triton X-100 buffer (1% Triton X-100, 25 mM Tris HCl) on ice for two hours and then microcentrifuged for 5 minutes. The soluble extract was retained (and was

labeled TX100). Cells were lysed in cell extraction buffer with freeze/thawing. The soluble cytoplasmic fraction was set aside (and labeled CEB). Nuclear pellets from the preparation of the CEB cytoplasmic fraction were solubilized with Triton X-100 buffer, microcentrifuged, and the soluble fractions, which contains primarily nuclear DNA, was retained (and labeled CEB-TX100). Soluble cell extract was prepared by lysing cells with NP-40 buffer, followed by microcentrifugation for 5 minutes (and was labeled NP-40). *In vitro* cleavage was performed by incubating 16 µl of each extract (CEB, TX-100, CEB-TX100, and NP-40) with 4 µl of *in vitro* translated XIAP protein at 37°C for 7 hours. Negative controls, containing only TX100 buffer or CEB buffer were also included. The proteins were separated on a 10% SDS-polyacrylamide gel, which was dried and exposed to X-ray film overnight.

In vitro cleavage of XIAP was apparent in the CEB extract. The observed molecular weight of the cleavage product was approximately 36 kDa (Fig. 23). The 10 kDa shift in the size of the cleavage product indicates that the observed product is derived from the amino-terminus of the recombinant protein, which contains six copies of the myc epitope (10 kDa). It thus appears that the cleavage product possesses at least two of the BIR domains, and that it is localized to the nucleus.

XIII. Treatment of HIV Infected Individuals

The expression of hiap-1 and hiap-2 is decreased significantly in HIV-infected human cells. Furthermore, this decrease precedes apoptosis. Therefore, administration of HIAP-1, HIAP-2, genes encoding these proteins, or compounds that upregulate these genes can be used to prevent T cell attrition in HIV-infected patients. The following assay may also be used to screen

for compounds that alter hiap-1 and hiap-2 expression, and which also prevent apoptosis.

Cultured mature lymphocyte CD-4$^+$ T cell lines (H9, labelled "a"; CEM/CM-3, labelled "b"; 6T-CEM, labelled "c"; and Jurkat, labelled "d" in Figs. 13A and 13B), were examined for signs of apoptosis (Fig. 13A) and hiap gene expression (Fig. 13B) after exposure to mitogens or HIV infection. Apoptosis was demonstrated by the appearance of DNA "laddering" upon gel electrophoresis and gene expression was assessed by PCR. The results obtained from normal (non-infected, non-mitogen stimulated) cells are shown in each lane labelled "1" in Figs. 13A and 13B. The results obtained 24 hours after PHA/PMA (phytohemagglutinin/phorbol ester) stimulation are shown in each lane labelled "2". The results obtained 24 hours after HIV strain III_B infection are shown in each lane labelled "3". The "M" refers to standard DNA markers (the 123 bp ladder in Fig. 13B, and the lambda HindIII ladder in Fig. 13A (both from Gibco-BRL)). DNA ladders (Prigent et al., J. Immunol. Methods, 160:139-140, 1993), which indicate apoptosis, are evident when DNA from the samples described above are electrophoresed on an ethidium bromide-stained agarose gel (Fig. 13A). The sensitivity and degree of apoptosis of the four T cell lines tested varies following mitogen stimulation and HIV infection.

In order to examine hiap gene expression, total RNA was prepared from the cultured cells and reverse transcribed using oligo-dT priming. The RT cDNA products were amplified by PCR using specific primers (as shown in Table 5) for the detection of hiap-2a, hiap-2b and hiap-1. The PCR was conducted using a PerkinElmer 480 thermocycler with 35 cycles of the following program: 94°C for one minute, 55°C for 2 minutes and 72°C for 1.5 minutes. The RT-PCR reaction products were

electrophoresed on a 1% agarose gel, which was stained with ethidium bromide. Absence of hiap-2 transcripts is noted in all four cell lines 24 hours after HIV infection. In three of four cell lines (all except H9), the hiap-1 gene is also dramatically down-regulated after HIV infection. PHA/PMA mitogen stimulation also appears to decrease hiap gene expression; particularly of hiap-2 and to a lesser extent, of hiap-1. The data from these experiments is summarized in Table 5. The expression of β-actin was consistent in all cell lines tested, indicating that there is not a flaw in the RT-PCR assay that could account for the decrease in hiap gene expression.

TABLE 4

OLIGONUCLEOTIDE PRIMERS FOR THE SPECIFIC RT-PCR AMPLIFICATION OF UNIQUE IAP GENES

IAP Gene	Forward Primer (nucleotide position*)	Reverse Primer (nucleotide position*)	Size of Product (bp)
h-xiap	p2415 (876-896)	p2449 (1291-1311)	435
m-xiap	p2566 (456-478)	p2490 (994-1013)	555
h-hiap1	p2465 (827-847)	p2464 (1008-1038)	211
m-hiap1	p2687 (747-767)	p2684 (1177-1197)	450
hiap2	p2595 (1562-1585)	p2578 (2339-2363)	801[a] 618[b]
m-hiap2	p2693 (1751-1772)	p2734 (2078-2100)	349

* Nucleotide position as determined from Figs. 1-4 for each IAP gene

[a] PCR product size of hiap2a

[b] PCR product size of hiap2b

TABLE 5

APOPTOSIS AND HIAP GENE EXPRESSION IN CULTURED T-CELLS FOLLOWING MITOGEN STIMULATION OR HIV INFECTION

Cell Line	Condition	Apoptosis	hiap1	hiap2
H9	not stimulated	-	+	±
	PHA/PMA stimulated	+++	+	±
	HIV infected	++	+	-
CEM/CM-3	not stimulated	-	+	±
	PHA/PMA stimulated	±	+	-
	HIV infected	±	-	-
6T-CEM	not stimulated	-	+	+
	PHA/PMA stimulated	±	-	-
	HIV infected	+	-	-
Jurkat	not stimulated	-	+	++
	PHA/PMA stimulated	+	+	+
	HIV infected	±	-	-

XIV. Assignment of xiap, hiap-1, and hiap-2 to Chromosomes Xq25 and 11q22-23 by Fluorescence *in situ* Hybridization (FISH)

Fluorescence *in situ* hybridization (FISH) was used to identify the chromosomal location of xiap, hiap-1 and hiap-2. The probes used were cDNAs cloned in plasmid vectors: the 2.4 kb xiap clone included 1493 bp of coding sequence, 34bp of 5' UTR (untranslated region) and 913 bp of 3'UTR; the hiap-1 cDNA was 3.1 kb long and included 1812 bp coding and 1300 bp of 3' UTR; and the hiap-2 clone consisted of 1856 bp of coding and 1200 bp of 5' UTR. A total of 1 μg of probe DNA was labelled with biotin by nick translation (BRL). Chromosome spreads prepared from a normal peripheral blood culture were denatured for 2 minutes at 70°C in 50% formamide/2X SSC and subsequently hybridized with the biotin labelled DNA probe for 18 hours at 37°C in a solution consisting of 2X SSC/70% formamide/10% dextran sulfate. After hybridization, the spreads were washed in 2X SSC/50% formamide, followed by a wash in 2X SSC at 42°C. The biotin labelled DNA was detected by fluorescein isothiocyanate (FITC) conjugated avidin antibodies and anti-avidin antibodies (ONCOR detection kit), according to the manufacturer's instructions. Chromosomes were counterstained with propidium iodide and examined with a Olympus BX60 epifluorescence microscope. For chromosome identification, the slides with recorded labelled metaphase spreads were destained, dehydrated, dried, digested with trypsin for 30 seconds and stained with 4% Giemsa stain for 2 minutes. The chromosome spreads were relocated and the images were compared.

A total of 101 metaphase spreads were examined with the xiap probe, as described above. Symmetrical fluorescent signals on either one or both homologs of

chromosome Xq25 were observed in 74% of the cells analyzed. Following staining with hiap-1 and hiap-2 probes, 56 cells were analyzed and doublet signals in the region 11q22-23 were observed in 83% of cells examined. The xiap gene was mapped to Xq25 while the hiap-1 and hiap-2 genes were mapped at the border of 11q22 and 11q23 bands.

These experiments confirmed the location of the xiap gene on chromosome Xq25. No highly consistent chromosomal abnormalities involving band Xq25 have been reported so far in any malignancies. However, deletions within this region are associated with a number of immune system defects including X-linked lymphoproliferative disease (Wu et al., Genomics 17:163-170, 1993).

Cytogenetic abnormalities of band 11q23 have been identified in more than 50% of infant leukemias regardless of the phenotype (Martinez-Climet et al., Leukaemia 9:1299-1304, 1995). Rearrangements of the MLL Gene (mixed lineage leukemia or myeloid lymphoid leukemia; Ziemin Van der Poel et al., Proc. Natl. Acad. Sci. USA 88:10735-10739, 1991) have been detected in 80% of cases with 11q23 translocation, however patients whose rearrangements clearly involved regions other than the MLL gene were also reported (Kobayashi et al., Blood 82:547-551, 1993). Thus, the IAP genes may follow the Bcl-2 paradigm, and would therefore play an important role in cancer transformation.

XV. Preventive Anti-Apoptotic Therapy

In a patient diagnosed to be heterozygous for an IAP mutation or to be susceptible to IAP mutations (even if those mutations do not yet result in alteration or loss of IAP biological activity), or a patient diagnosed as HIV positive, any of the above therapies may be administered before the occurrence of the disease

phenotype. For example, the therapies may be provided to a patient who is HIV positive but does not yet show a diminished T cell count or other overt signs of AIDS. In particular, compounds shown to increase IAP expression or IAP biological activity may be administered by any standard dosage and route of administration (see above). Alternatively, gene therapy using an IAP expression construct may be undertaken to reverse or prevent the cell defect prior to the development of the degenerative disease.

The methods of the instant invention may be used to reduce or diagnose the disorders described herein in any mammal, for example, humans, domestic pets, or livestock. Where a non-human mammal is treated or diagnosed, the IAP polypeptide, nucleic acid, or antibody employed is preferably specific for that species.

Other Embodiments

In other embodiments, the invention includes any protein which is substantially identical to a mammalian IAP polypeptides (Figs. 1-6; SEQ ID NOs:1-42); such homologs include other substantially pure naturally-occurring mammalian IAP proteins as well as allelic variants; natural mutants; induced mutants; DNA sequences which encode proteins and also hybridize to the IAP DNA sequences of Figs. 1-6 (SEQ ID NOS:1-42) under high stringency conditions or, less preferably, under low stringency conditions (e.g., washing at 2X SSC at 400C with a probe length of at least 40 nucleotides); and proteins specifically bound by antisera directed to a IAP polypeptide. The term also includes chimeric polypeptides that include a IAP portion.

The invention further includes analogs of any naturally-occurring IAP polypeptide. Analogs can differ from the naturally-occurring IAP protein by amino acid

sequence differences, by post-translational modifications, or by both. Analogs of the invention will generally exhibit at least 85%, more preferably 90%, and most preferably 95% or even 99% identity with all or part of a naturally occurring IAP amino acid sequence. The length of sequence comparison is at least 15 amino acid residues, preferably at least 25 amino acid residues, and more preferably more than 35 amino acid residues. Modifications include *in vivo* and *in vitro* chemical derivatization of polypeptides, e.g., acetylation, carboxylation, phosphorylation, or glycosylation; such modifications may occur during polypeptide synthesis or processing or following treatment with isolated modifying enzymes. Analogs can also differ from the naturally-occurring IAP polypeptide by alterations in primary sequence. These include genetic variants, both natural and induced (for example, resulting from random mutagenesis by irradiation or exposure to ethanemethylsulfate or by site-specific mutagenesis as described in Sambrook, Fritsch and Maniatis, Molecular Cloning: A Laboratory Manual (2d ed.), CSH Press, 1989, or Ausubel et al., *supra*). Also included are cyclized peptides, molecules, and analogs which contain residues other than L-amino acids, e.g., D-amino acids or non-naturally occurring or synthetic amino acids, e.g., B or y amino acids. In addition to full-length polypeptides, the invention also includes IAP polypeptide fragments. As used herein, the term "fragment," means at least 20 contiguous amino acids, preferably at least 30 contiguous amino acids, more preferably at least 50 contiguous amino acids, and most preferably at least 60 to 80 or more contiguous amino acids. Fragments of IAP polypeptides can be generated by methods known to those skilled in the art or may result from normal protein processing (e.g., removal of amino acids from the nascent polypeptide that

are not required for biological activity or removal of amino acids by alternative mRNA splicing or alternative protein processing events).

Preferable fragments or analogs according to the invention are those which facilitate specific detection of a IAP nucleic acid or amino acid sequence in a sample to be diagnosed. Particularly useful IAP fragments for this purpose include, without limitation, the amino acid fragments shown in Table 2.

What is claimed is:

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: University of Ottawa
Korneluk, Robert G.
Mackenzie, Alexander E.
Baird, Stephen
Liston, Peter

(ii) TITLE OF INVENTION: MAMMALIAN IAP GENE FAMILY, PRIMERS, PROBES, AND DETECTION METHODS

(iii) NUMBER OF SEQUENCES: 45

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(F) ZIP: 02110-2804

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/IB96/----
(B) FILING DATE: 05-AUG-1996
(C) CLASSIFICATION:

(vii) PRIORITY APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/511,485
(B) FILING DATE: 04-AUG-1995
(C) CLASSIFICATION:

(vii) PRIORITY APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/576,956
(B) FILING DATE: 22-DEC-1995
(C) CLASSIFICATION:

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(ix) TELECOMMUNICATION INFORMATION:
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(C) TELEX: 200154

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(D) OTHER INFORMATION: Xaa at positons 2, 3, 4, 5, 6, 7, 9, 10, 11, 17, 18, 19, 20, 21, 23, 25, 30, 31, 32, 34, 35, 38, 39, 40, 41, 42, and 45 may be any amino acid. Xaa at positon 8 is Glu or Asp. Xaa at positions 14 & 22 is Val or Ile.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Glu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Lys Xaa Cys Met
1 5 10 15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Phe Xaa Pro Cys Gly His Xaa Xaa Xaa
20 25 30

Cys Xaa Xaa Cys Ala Xaa Xaa Xaa Xaa Xaa Cys Pro Xaa Cys
35 40 45

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(D) OTHER INFORMATION: Xaa at positions 1, 2, 3, 6, 9, 10, 14, 15, 18, 19, 20, 21, 24, 30, 32, 33, 35, 37, 40, 42, 43, 44, 45, 46, 47, 49, 50, 51, 53, 54, 55, 56, 57, 59, 60, 61, 62, 64 and 66 may be any amino acid. Xaa at positions 13, 16 and 17 may be any amino acid or may be absent.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Xaa Xaa Xaa Arg Leu Xaa Thr Phe Xaa Xaa Trp Pro Xaa Xaa Xaa Xaa
1 5 10 15

Xaa Xaa Xaa Xaa Xaa Leu Ala Xaa Ala Gly Phe Tyr Tyr Xaa Gly Xaa
20 25 30

Xaa Asp Xaa Val Xaa Cys Phe Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Trp
35 40 45

Xaa Xaa Xaa Asp Xaa Xaa Xaa Xaa Xaa His Xaa Xaa Xaa Xaa Pro Xaa
50 55 60

Cys Xaa Phe Val
65

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2540 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAAAAGGTGG ACAAGTCCTA TTTTCAAGAG AAGATGACTT TTAACAGTTT TGAAGGATCT 60

AAAACTTGTG TACCTGCAGA CATCAATAAG GAAGAAGAAT TTGTAGAAGA GTTTAATAGA 120

TTAAAAACTT TTGCTAATTT TCCAAGTGGT AGTCCTGTTT CAGCATCAAC ACTGGCACGA 180

GCAGGGTTTC TTTATACTGG TGAAGGAGAT ACCGTGCGGT GCTTTAGTTG TCATGCAGCT 240

GTAGATAGAT GGCAATATGG AGACTCAGCA GTTGGAAGAC ACAGGAAAGT ATCCCCAAAT 300

TGCAGATTTA TCAACGGCTT TTATCTTGAA AATAGTGCCA CGCAGTCTAC AAATTCTGGT 360

ATCCAGAATG GTCAGTACAA AGTTGAAAAC TATCTGGGAA GCAGAGATCA TTTTGCCTTA 420

GACAGGCCAT CTGAGACACA TGCAGACTAT CTTTTCAGAA CTGGGCAGGT TGTAGATATA 480

TCAGACACCA TATACCCGAG GAACCCTGCC ATGTATTGTG AAGAAGCTAG ATTAAAGTCC 540

TTTCAGAACT GGCCAGACTA TGCTCACCTA ACCCCAAGAG AGTTAGCAAG TGCTGGACTC 600

TACTACACAG GTATTGGTGA CCAAGTGCAG TGCTTTTGTT GTGGTGGAAA ACTGAAAAAT 660

TGGGAACCTT GTGATCGTGC CTGGTCAGAA CACAGGCGAC ACTTTCCTAA TTGCTTCTTT 720

GTTTTGGGCC GGAATCTTAA TATTCGAAGT GAATCTGATG CTGTGAGTTC TGATAGGAAT 780

TTCCCAAATT CAACAAATCT TCCAAGAAAT CCATCCATGG CAGATTATGA AGCACGGATC 840

TTTACTTTTG GGACATGGAT ATACTCAGTT AACAAGGAGC AGCTTGCAAG AGCTGGATTT 900

TATGCTTTAG GTGAAGGTGA TAAAGTAAAG TGCTTTCACT GTGGAGGAGG GCTAACTGAT 960

TGGAAGCCCA GTGAAGACCC TTGGGAACAA CATGCTAAAT GGTATCCAGG GTGCAAATAT 1020

CTGTTAGAAC AGAAGGGACA AGAATATATA AACAATATTC ATTTAACTCA TTCACTTGAG 1080

GAGTGTCTGG TAAGAACTAC TGAGAAAACA CCATCACTAA CTAGAAGAAT TGATGATACC 1140

ATCTTCCAAA ATCCTATGGT ACAAGAAGCT ATACGAATGG GGTTCAGTTT CAAGGACATT 1200

AAGAAAATAA TGGAGGAAAA AATTCAGATA TCTGGGAGCA ACTATAAATC ACTTGAGGTT 1260

CTGGTTGCAG ATCTAGTGAA TGCTCAGAAA GACAGTATGC AAGATGAGTC AAGTCAGACT 1320

TCATTACAGA AAGAGATTAG TACTGAAGAG CAGCTAAGGC GCCTGCAAGA GGAGAAGCTT 1380

TCCAAAATCT GTATGGATAG AAATATTGCT ATCGTTTTTG TTCCTTGTGG ACATCTAGTC 1440

ACTTGTAAAC AATGTGCTGA AGCAGTTGAC AAGTGTCCCA TGTGCTACAC AGTCATTACT 1500

TTCAAGCAAA AAATTTTTAT GTCTTAATCT AACTCTATAG TAGGCATGTT ATGTTGTTCT 1560

TATTACCCTG ATTGAATGTG TGATGTGAAC TGACTTTAAG TAATCAGGAT TGAATTCCAT 1620

TAGCATTTGC TACCAAGTAG GAAAAAAAAT GTACATGGCA GTGTTTTAGT TGGCAATATA 1680

ATCTTTGAAT TTCTTGATTT TTCAGCGTAT TAGCTGTATT ATCCATTTTT TTTACTGTTA 1740

TTTAATTGAA ACCATAGACT AAGAATAAGA AGCATCATAC TATAACTGAA CACAATGTGT 1800

ATTCATAGTA TACTGATTTA ATTTCTAAGT GTAAGTGAAT TAATCATCTG GATTTTTTAT 1860

TCTTTTCAGA TAGGCTTAAC AAATGGAGCT TTCTGTATAT AAATGTGGAG ATTAGAGTTA 1920

ATCTCCCCAA TCACATAATT TGTTTTGTGT GAAAAAGGAA TAAATTGTTC CATGCTGGTG 1980

GAAAGATAGA GATTGTTTTT AGAGGTTGGT TGTTGTGTTT TAGGATTCTG TCCATTTTCT 2040

TGTAAAGGGA TAAACACGGA CGTGTGCGAA ATATGTTTGT AAAGTGATTT GCCATTGTTG 2100

AAAGCGTATT TAATGATAGA ATACTATCGA GCCAACATGT ACTGACATGG AAAGATGTCA 2160

GAGATATGTT AAGTGTAAAA TGCAAGTGGC GGGACACTAT GTATAGTCTG AGCCAGATCA 2220

AAGTATGTAT GTTGTTAATA TGCATAGAAC GAGAGATTTG GAAAGATATA CACCAAACTG 2280

TTAAATGTGG TTTCTCTTCG GGGAGGGGGG GATTGGGGGA GGGGCCCCAG AGGGGTTTTA 2340

GAGGGGCCTT TTCACTTTCG ACTTTTTTCA TTTTGTTCTG TTCGGATTTT TTATAAGTAT 2400

GTAGACCCCG AAGGGTTTTA TGGGAACTAA CATCAGTAAC CTAACCCCCG TGACTATCCT 2460

GTGCTCTTCC TAGGGAGCTG TGTTGTTTCC CACCCACCAC CCTTCCCTCT GAACAAATGC 2520

CTGAGTGCTG GGGCACTTTN 2540

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 497 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Thr Phe Asn Ser Phe Glu Gly Ser Lys Thr Cys Val Pro Ala Asp
1 5 10 15

Ile Asn Lys Glu Glu Glu Phe Val Glu Glu Phe Asn Arg Leu Lys Thr
20 25 30

Phe Ala Asn Phe Pro Ser Gly Ser Pro Val Ser Ala Ser Thr Leu Ala
35 40 45

Arg Ala Gly Phe Leu Tyr Thr Gly Glu Gly Asp Thr Val Arg Cys Phe
50 55 60

Ser Cys His Ala Ala Val Asp Arg Trp Gln Tyr Gly Asp Ser Ala Val
65 70 75 80

Gly Arg His Arg Lys Val Ser Pro Asn Cys Arg Phe Ile Asn Gly Phe
85 90 95

Tyr Leu Glu Asn Ser Ala Thr Gln Ser Thr Asn Ser Gly Ile Gln Asn
100 105 110

Gly Gln Tyr Lys Val Glu Asn Tyr Leu Gly Ser Arg Asp His Phe Ala
115 120 125

Leu Asp Arg Pro Ser Glu Thr His Ala Asp Tyr Leu Leu Arg Thr Gly
130 135 140

Gln Val Val Asp Ile Ser Asp Thr Ile Tyr Pro Arg Asn Pro Ala Met
145 150 155 160

Tyr Cys Glu Glu Ala Arg Leu Lys Ser Phe Gln Asn Trp Pro Asp Tyr
165 170 175

Ala His Leu Thr Pro Arg Glu Leu Ala Ser Ala Gly Leu Tyr Tyr Thr
180 185 190

Gly Ile Gly Asp Gln Val Gln Cys Phe Cys Cys Gly Gly Lys Leu Lys
195 200 205

Asn Trp Glu Pro Cys Asp Arg Ala Trp Ser Glu His Arg Arg His Phe
210 215 220

Pro Asn Cys Phe Phe Val Leu Gly Arg Asn Leu Asn Ile Arg Ser Glu
225 230 235 240

Ser Asp Ala Val Ser Ser Asp Arg Asn Phe Pro Asn Ser Thr Asn Leu
245 250 255

Pro Arg Asn Pro Ser Met Ala Asp Tyr Glu Ala Arg Ile Phe Thr Phe
260 265 270

Gly Thr Trp Ile Tyr Ser Val Asn Lys Glu Gln Leu Ala Arg Ala Gly
275 280 285

Phe Tyr Ala Leu Gly Glu Gly Asp Lys Val Lys Cys Phe His Cys Gly
290 295 300

Gly Gly Leu Thr Asp Trp Lys Pro Ser Glu Asp Pro Trp Glu Gln His
305 310 315 320

Ala Lys Trp Tyr Pro Gly Cys Lys Tyr Leu Leu Glu Gln Lys Gly Gln
325 330 335

Glu Tyr Ile Asn Asn Ile His Leu Thr His Ser Leu Glu Glu Cys Leu
340 345 350

Val Arg Thr Thr Glu Lys Thr Pro Ser Leu Thr Arg Arg Ile Asp Asp
355 360 365

Thr Ile Phe Gln Asn Pro Met Val Gln Glu Ala Ile Arg Met Gly Phe
370 375 380

Ser Phe Lys Asp Ile Lys Lys Ile Met Glu Glu Lys Ile Gln Ile Ser
385 390 395 400

Gly Ser Asn Tyr Lys Ser Leu Glu Val Leu Val Ala Asp Leu Val Asn
405 410 415

Ala Gln Lys Asp Ser Met Gln Asp Glu Ser Ser Gln Thr Ser Leu Gln
420 425 430

Lys Glu Ile Ser Thr Glu Glu Gln Leu Arg Arg Leu Gln Glu Glu Lys
435 440 445

Leu Cys Lys Ile Cys Met Asp Arg Asn Ile Ala Ile Val Phe Val Pro
450 455 460

Cys Gly His Leu Val Thr Cys Lys Gln Cys Ala Glu Ala Val Asp Lys
465 470 475 480

Cys Pro Met Cys Tyr Thr Val Ile Thr Phe Lys Gln Lys Ile Phe Met
485 490 495

Ser

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2676 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TCCTTGAGAT GTATCAGTAT ACGATTTAGG ATCTCCATGT TGGAACTCTA AATGCATAGA 60

AATGGAAATA ATGGAAATTT TTCATTTTGG CTTTTCAGCC TAGTATTAAA ACTGATAAAA 120

GCAAAGCCAT GCACAAAACT ACCTCCCTAG AGAAAGGCTA GTCCCTTTTC TTCCCCATTC 180

ATTTCATTAT GAACATAGTA GAAAACAGCA TATTCTTATC AAATTTGATG AAAAGCGCCA 240

ACACGTTTGA ACTGAAATAC GACTTGTCAT GTGAACTGTA CCGAATGTCT ACGTATTCCA 300

CTTTTCCTGC TGGGGTTCCT GTCTCAGAAA GGACTCTTGC TCGTCCTGCT TTCTATTACA 360

CTGGTGTGAA TGACAAGGTC AAATGCTTCT GTTGTGGCCT GATGCTGGAT AACTGGAAAA 420

GAGGAGACAG TCCTACTGAA AAGCATAAAA AGTTGTATCC TAGCTGCAGA TTCGTTCAGA 480

GTCTAAATTC CGTTAACAAC TTGGAAGCTA CCTCTCAGCC TACTTTTCCT TCTTCAGTAA 540

CACATTCCAC ACACTCATTA CTTCCGGGTA CAGAAAACAG TGGATATTTC CGTGGCTCTT 600

ATTCAAACTC TCCATCAAAT CCTGTAAACT CCAGAGCAAA TCAAGAATTT TCTGCCTTGA 660

TGAGAAGTTC CTACCCCTGT CCAATGAATA ACGAAAATGC CAGATTACTT ACTTTTCAGA 720

CATGGCCATT GACTTTTCTG TCGCCAACAG ATCTGGCACG AGCAGGCTTT TACTACATAG 780

GACCTGGAGA CAGAGTGGCT TGCTTTGCCT GTGGTGGAAA ATTGAGCAAT TGGGAACCGA 840

AGGATAATGC TATGTCAGAA CACCTGAGAC ATTTTCCCAA ATGCCCATTT ATAGAAAATC 900

AGCTTCAAGA CACTTCAAGA TACACAGTTT CTAATCTGAG CATGCAGACA CATGCAGCCC 960

GCTTTAAAAC ATTCTTTAAC TGGCCCTCTA GTGTTCTAGT TAATCCTGAG CAGCTTGCAA 1020

GTGCGGGTTT TTATTATGTG GGTAACAGTG ATGATGTCAA ATGCTTTTGC TGTGATGGTG 1080

GACTCAGGTG TTGGGAATCT GGAGATGATC CATGGGTTCA ACATGCCAAG TGGTTTCCAA 1140

GGTGTGAGTA CTTGATAAGA ATTAAAGGAC AGGAGTTCAT CCGTCAAGTT CAAGCCAGTT 1200

ACCCTCATCT ACTTGAACAG CTGCTATCCA CATCAGACAG CCCAGGAGAT GAAAATGCAG 1260

AGTCATCAAT TATCCATTTG GAACCTGGAG AAGACCATTC AGAAGATGCA ATCATGATGA 1320

ATACTCCTGT GATTAATGCT GCCGTGGAAA TGGGCTTTAG TAGAAGCCTG GTAAAACAGA 1380

CAGTTCAGAG AAAAATCCTA GCAACTGGAG AGAATTATAG ACTAGTCAAT GATCTTGTGT 1440

TAGACTTACT CAATGCAGAA GATGAAATAA GGGAAGAGGA GAGAGAAAGA GCAACTGAGG 1500

AAAAAGAATC AAATGATTTA TTATTAATCC GGAAGAATAG AATGGCACTT TTTCAACATT 1560

TGACTTGTGT AATTCCAATC CTGGATAGTC TACTAACTGC CGGAATTATT AATGAACAAG 1620

AACATGATGT TATTAAACAG AAGACACAGA CGTCTTTACA AGCAAGAGAA CTGATTGATA 1680

CGATTTTAGT AAAAGGAAAT ATTGCAGCCA CTGTATTCAG AAACTCTCTG CAAGAAGCTG 1740

AAGCTGTGTT ATATGAGCAT TTATTTGTGC AACAGGACAT AAAATATATT CCCACAGAAG 1800

ATGTTTCAGA TCTACCAGTG GAAGAACAAT TGCGGAGACT ACCAGAAGAA AGAACATGTA 1860

AAGTGTGTAT GGACAAAGAA GTGTCCATAG TGTTTATTCC TTGTGGTCAT CTAGTAGTAT 1920

GCAAAGATTG TGCTCCTTCT TTAAGAAAGT GTCCTATTTG TAGGAGTACA ATCAAGGGTA 1980

CAGTTCGTAC ATTTCTTTCA TGAAGAAGAA CCAAAACATC GTCTAAACTT TAGAATTAAT 2040

TTATTAAATG TATTATAACT TTAACTTTTA TCCTAATTTG GTTTCCTTAA AATTTTTATT 2100

TATTTACAAC TCAAAAAACA TTGTTTTGTG TAACATATTT ATATATGTAT CTAAACCATA 2160

TGAACATATA TTTTTTAGAA ACTAAGAGAA TGATAGGCTT TTGTTCTTAT GAACGAAAAA 2220

GAGGTAGCAC TACAAACACA ATATTCAATC CAAATTTCAG CATTATTGAA ATTGTAAGTG 2280

AAGTAAAACT TAAGATATTT GAGTTAACCT TTAAGAATTT TAAATATTTT GGCATTGTAC 2340

TAATACCGGG AACATGAAGC CAGGTGTGGT GGTATGTACC TGTAGTCCCA GGCTGAGGCA 2400

AGAGAATTAC TTGAGCCCAG GAGTTTGAAT CCATCCTGGG CAGCATACTG AGACCCTGCC 2460

TTTAAAAACN AACAGNACCA AANCCAAACA CCAGGGACAC ATTTCTCTGT CTTTTTTGAT 2520

CAGTGTCCTA TACATCGAAG GTGTGCATAT ATGTTGAATC ACATTTTAGG GACATGGTGT 2580

TTTTATAAAG AATTCTGTGA GNAAAAATTT AATAAAGCAA CCAAATTACT CTTAAAAAAA 2640

AAAAAAAAAA AAAAAACTCG AGGGGCCCGT ACCAAT 2676

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 604 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Asn Ile Val Glu Asn Ser Ile Phe Leu Ser Asn Leu Met Lys Ser
1 5 10 15

Ala Asn Thr Phe Glu Leu Lys Tyr Asp Leu Ser Cys Glu Leu Tyr Arg
20 25 30

Met Ser Thr Tyr Ser Thr Phe Pro Ala Gly Val Pro Val Ser Glu Arg
35 40 45

Ser Leu Ala Arg Ala Gly Phe Tyr Tyr Thr Gly Val Asn Asp Lys Val
50 55 60

Lys Cys Phe Cys Cys Gly Leu Met Leu Asp Asn Trp Lys Arg Gly Asp
65 70 75 80

Ser Pro Thr Glu Lys His Lys Lys Leu Tyr Pro Ser Cys Arg Phe Val
85 90 95

Gln Ser Leu Asn Ser Val Asn Asn Leu Glu Ala Thr Ser Gln Pro Thr
100 105 110

Phe Pro Ser Ser Val Thr His Ser Thr His Ser Leu Leu Pro Gly Thr
115 120 125

Glu Asn Ser Gly Tyr Phe Arg Gly Ser Tyr Ser Asn Ser Pro Ser Asn
130 135 140

Pro Val Asn Ser Arg Ala Asn Gln Glu Phe Ser Ala Leu Met Arg Ser
145 150 155 160

Ser Tyr Pro Cys Pro Met Asn Asn Glu Asn Ala Arg Leu Leu Thr Phe
165 170 175

Gln Thr Trp Pro Leu Thr Phe Leu Ser Pro Thr Asp Leu Ala Arg Ala
180 185 190

Gly Phe Tyr Tyr Ile Gly Pro Gly Asp Arg Val Ala Cys Phe Ala Cys
195 200 205

Gly Gly Lys Leu Ser Asn Trp Glu Pro Lys Asp Asn Ala Met Ser Glu
210 215 220

His Leu Arg His Phe Pro Lys Cys Pro Phe Ile Glu Asn Gln Leu Gln
225 230 235 240

Asp Thr Ser Arg Tyr Thr Val Ser Asn Leu Ser Met Gln Thr His Ala
245 250 255

Ala Arg Phe Lys Thr Phe Phe Asn Trp Pro Ser Ser Val Leu Val Asn
260 265 270

Pro Glu Gln Leu Ala Ser Ala Gly Phe Tyr Tyr Val Gly Asn Ser Asp
275 280 285

Asp Val Lys Cys Phe Cys Cys Asp Gly Gly Leu Arg Cys Trp Glu Ser
290 295 300

Gly Asp Asp Pro Trp Val Gln His Ala Lys Trp Phe Pro Arg Cys Glu
305 310 315 320

Tyr Leu Ile Arg Ile Lys Gly Gln Glu Phe Ile Arg Gln Val Gln Ala
325 330 335

Ser Tyr Pro His Leu Leu Glu Gln Leu Leu Ser Thr Ser Asp Ser Pro
340 345 350

Gly Asp Glu Asn Ala Glu Ser Ser Ile Ile His Leu Glu Pro Gly Glu
355 360 365

Asp His Ser Glu Asp Ala Ile Met Met Asn Thr Pro Val Ile Asn Ala
370 375 380

Ala Val Glu Met Gly Phe Ser Arg Ser Leu Val Lys Gln Thr Val Gln
385 390 395 400

Arg Lys Ile Leu Ala Thr Gly Glu Asn Tyr Arg Leu Val Asn Asp Leu
405 410 415

Val Leu Asp Leu Leu Asn Ala Glu Asp Glu Ile Arg Glu Glu Glu Arg
420 425 430

Glu Arg Ala Thr Glu Glu Lys Glu Ser Asn Asp Leu Leu Leu Ile Arg
435 440 445

Lys Asn Arg Met Ala Leu Phe Gln His Leu Thr Cys Val Ile Pro Ile
450 455 460

Leu Asp Ser Leu Leu Thr Ala Gly Ile Ile Asn Glu Gln Glu His Asp
465 470 475 480

Val Ile Lys Gln Lys Thr Gln Thr Ser Leu Gln Ala Arg Glu Leu Ile
485 490 495

Asp Thr Ile Leu Val Lys Gly Asn Ile Ala Ala Thr Val Phe Arg Asn
500 505 510

Ser Leu Gln Glu Ala Glu Ala Val Leu Tyr Glu His Leu Phe Val Gln
515 520 525

Gln Asp Ile Lys Tyr Ile Pro Thr Glu Asp Val Ser Asp Leu Pro Val
530 535 540

Glu Glu Gln Leu Arg Arg Leu Pro Glu Glu Arg Thr Cys Lys Val Cys
545 550 555 560

Met Asp Lys Glu Val Ser Ile Val Phe Ile Pro Cys Gly His Leu Val
565 570 575

Val Cys Lys Asp Cys Ala Pro Ser Leu Arg Lys Cys Pro Ile Cys Arg
580 585 590

Ser Thr Ile Lys Gly Thr Val Arg Thr Phe Leu Ser
595 600

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2580 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TTAGGTTACC TGAAAGAGTT ACTACAACCC CAAAGAGTTG TGTTCTAAGT AGTATCTTGG 60

TAATTCAGAG AGATACTCAT CCTACCTGAA TATAAACTGA GATAAATCCA GTAAAGAAAG 120

TGTAGTAAAT TCTACATAAG AGTCTATCAT TGATTTCTTT TTGTGGTGGA AATCTTAGTT 180

CATGTGAAGA AATTTCATGT GAATGTTTTA GCTATCAAAC AGTACTGTCA CCTACTCATG 240

CACAAAACTG CCTCCCAAAG ACTTTTCCCA GGTCCCTCGT ATCAAAACAT TAAGAGTATA 300

ATGGAAGATA GCACGATCTT GTCAGATTGG ACAAACAGCA ACAAACAAAA AATGAAGTAT 360

GACTTTTCCT GTGAACTCTA CAGAATGTCT ACATATTCAA CTTTCCCCGC CGGGGTGCCT 420

GTCTCAGAAA GGAGTCTTGC TCGTGCTGGT TTTTATTATA CTGGTGTGAA TGACAAGGTC 480

AAATGCTTCT GTTGTGGCCT GATGCTGGAT AACTGGAAAC TAGGAGACAG TCCTATTCAA 540

AAGCATAAAC AGCTATATCC TAGCTGTAGC TTTATTCAGA ATCTGGTTTC AGCTAGTCTG 600

GGATCCACCT CTAAGAATAC GTCTCCAATG AGAAACAGTT TTGCACATTC ATTATCTCCC 660

ACCTTGGAAC ATAGTAGCTT GTTCAGTGGT TCTTACTCCA GCCTTCCTCC AAACCCTCTT 720

AATTCTAGAG CAGTTGAAGA CATCTCTTCA TCGAGGACTA ACCCCTACAG TTATGCAATG 780

AGTACTGAAG AAGCCAGATT TCTTACCTAC CATATGTGGC CATTAACTTT TTTGTCACCA 840

TCAGAATTGG CAAGAGCTGG TTTTTATTAT ATAGGACCTG GAGATAGGGT AGCCTGCTTT 900

GCCTGTGGTG GGAAGCTCAG TAACTGGGAA CCAAAGGATG ATGCTATGTC AGAACACCGG 960

AGGCATTTTC CCAACTGTCC ATTTTTGGAA AATTCTCTAG AAACTCTGAG GTTTAGCATT 1020

TCAAATCTGA GCATGCAGAC ACATGCAGCT CGAATGAGAA CATTTATGTA CTGGCCATCT 1080

AGTGTTCCAG TTCAGCCTGA GCAGCTTGCA AGTGCTGGTT TTTATTATGT GGGTCGCAAT 1140

GATGATGTCA AATGCTTTGG TTGTGATGGT GGCTTGAGGT GTTGGGAATC TGGAGATGAT 1200

CCATGGGTAG AACATGCCAA GTGGTTTCCA AGGTGTGAGT TCTTGATACG AATGAAAGGC 1260

CAAGAGTTTG TTGATGAGAT TCAAGGTAGA TATCCTCATC TTCTTGAACA GCTGTTGTCA 1320

ACTTCAGATA CCACTGGAGA AGAAAATGCT GACCCACCAA TTATTCATTT TGGACCTGGA 1380

GAAAGTTCTT CAGAAGATGC TGTCATGATG AATACACCTG TGGTTAAATC TGCCTTGGAA 1440

ATGGGCTTTA ATAGAGACCT GGTGAAACAA ACAGTTCTAA GTAAAATCCT GACAACTGGA 1500

GAGAACTATA AAACAGTTAA TGATATTGTG TCAGCACTTC TTAATGCTGA AGATGAAAAA 1560

AGAGAAGAGG AGAAGGAAAA ACAAGCTGAA GAAATGGCAT CAGATGATTT GTCATTAATT 1620

CGGAAGAACA GAATGGCTCT CTTTCAACAA TTGACATGTG TGCTTCCTAT CCTGGATAAT 1680

CTTTTAAAGG CCAATGTAAT TAATAAACAG GAACATGATA TTATTAAACA AAAAACACAG 1740

ATACCTTTAC AAGCGAGAGA ACTGATTGAT ACCATTTGGG TTAAAGGAAA TGCTGCGGCC 1800

AACATCTTCA AAAACTGTCT AAAAGAAATT GACTCTACAT TGTATAAGAA CTTATTTGTG 1860

GATAAGAATA TGAAGTATAT TCCAACAGAA GATGTTTCAG GTCTGTCACT GGAAGAACAA 1920

TTGAGGAGGT TGCAAGAAGA ACGAACTTGT AAAGTGTGTA TGGACAAAGA AGTTTCTGTT 1980

GTATTTATTC CTTGTGGTCA TCTGGTAGTA TGCCAGGAAT GTGCCCCTTC TCTAAGAAAA 2040

TGCCCTATTT GCAGGGGTAT AATCAAGGGT ACTGTTCGTA CATTTCTCTC TTAAAGAAAA 2100

ATAGTCTATA TTTTAACCTG CATAAAAAGG TCTTTAAAAT ATTGTTGAAC ACTTGAAGCC 2160

ATCTAAAGTA AAAAGGGAAT TATGAGTTTT TCAATTAGTA ACATTCATGT TCTAGTCTGC 2220

TTTGGTACTA ATAATCTTGT TTCTGAAAAG ATGGTATCAT ATATTTAATC TTAATCTGTT 2280

TATTTACAAG GGAAGATTTA TGTTTGGTGA ACTATATTAG TATGTATGTG TACCTAAGGG 2340

AGTAGCGTCN CTGCTTGTTA TGCATCATTT CAGGAGTTAC TGGATTTGTT GTTCTTTCAG 2400

AAAGCTTTGA ANACTAAATT ATAGTGTAGA AAAGAACTGG AAACCAGGAA CTCTGGAGTT 2460

CATCAGAGTT ATGGTGCCGA ATTGTCTTTG GTGCTTTTCA CTTGTGTTTT AAAATAAGGA 2520

TTTTTCTCTT ATTTCTCCCC CTAGTTTGTG AGAAACATCT CAATAAAGTG CTTTAAAAAG 2580

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 618 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met His Lys Thr Ala Ser Gln Arg Leu Phe Pro Gly Pro Ser Tyr Gln
1 5 10 15

Asn Ile Lys Ser Ile Met Glu Asp Ser Thr Ile Leu Ser Asp Trp Thr
20 25 30

Asn Ser Asn Lys Gln Lys Met Lys Tyr Asp Phe Ser Cys Glu Leu Tyr
35 40 45

Arg Met Ser Thr Tyr Ser Thr Phe Pro Ala Gly Val Pro Val Ser Glu
50 55 60

Arg Ser Leu Ala Arg Ala Gly Phe Tyr Tyr Thr Gly Val Asn Asp Lys
65 70 75 80

Val Lys Cys Phe Cys Cys Gly Leu Met Leu Asp Asn Trp Lys Leu Gly
85 90 95

Asp Ser Pro Ile Gln Lys His Lys Gln Leu Tyr Pro Ser Cys Ser Phe
100 105 110

Ile Gln Asn Leu Val Ser Ala Ser Leu Gly Ser Thr Ser Lys Asn Thr
115 120 125

Ser Pro Met Arg Asn Ser Phe Ala His Ser Leu Ser Pro Thr Leu Glu
130 135 140

His Ser Ser Leu Phe Ser Gly Ser Tyr Ser Ser Leu Pro Pro Asn Pro
145 150 155 160

Leu Asn Ser Arg Ala Val Glu Asp Ile Ser Ser Ser Arg Thr Asn Pro
165 170 175

Tyr Ser Tyr Ala Met Ser Thr Glu Glu Ala Arg Phe Leu Thr Tyr His
180 185 190

Met Trp Pro Leu Thr Phe Leu Ser Pro Ser Glu Leu Ala Arg Ala Gly
195 200 205

Phe Tyr Tyr Ile Gly Pro Gly Asp Arg Val Ala Cys Phe Ala Cys Gly
210 215 220

Gly Lys Leu Ser Asn Trp Glu Pro Lys Asp Asp Ala Met Ser Glu His
225 230 235 240

Arg Arg His Phe Pro Asn Cys Pro Phe Leu Glu Asn Ser Leu Glu Thr
245 250 255

Leu Arg Phe Ser Ile Ser Asn Leu Ser Met Gln Thr His Ala Ala Arg
260 265 270

Met Arg Thr Phe Met Tyr Trp Pro Ser Ser Val Pro Val Gln Pro Glu
275 280 285

Gln Leu Ala Ser Ala Gly Phe Tyr Tyr Val Gly Arg Asn Asp Asp Val
290 295 300

Lys Cys Phe Gly Cys Asp Gly Gly Leu Arg Cys Trp Glu Ser Gly Asp
305 310 315 320

Asp Pro Trp Val Glu His Ala Lys Trp Phe Pro Arg Cys Glu Phe Leu
325 330 335

Ile Arg Met Lys Gly Gln Glu Phe Val Asp Glu Ile Gln Gly Arg Tyr
340 345 350

Pro His Leu Leu Glu Gln Leu Leu Ser Thr Ser Asp Thr Thr Gly Glu
355 360 365

Glu Asn Ala Asp Pro Pro Ile Ile His Phe Gly Pro Gly Glu Ser Ser
370 375 380

Ser Glu Asp Ala Val Met Met Asn Thr Pro Val Val Lys Ser Ala Leu
385 390 395 400

Glu Met Gly Phe Asn Arg Asp Leu Val Lys Gln Thr Val Leu Ser Lys
405 410 415

Ile Leu Thr Thr Gly Glu Asn Tyr Lys Thr Val Asn Asp Ile Val Ser
420 425 430

Ala Leu Leu Asn Ala Glu Asp Glu Lys Arg Glu Glu Glu Lys Glu Lys
435 440 445

Gln Ala Glu Glu Met Ala Ser Asp Asp Leu Ser Leu Ile Arg Lys Asn
450 455 460

Arg Met Ala Leu Phe Gln Gln Leu Thr Cys Val Leu Pro Ile Leu Asp
465 470 475 480

Asn Leu Leu Lys Ala Asn Val Ile Asn Lys Gln Glu His Asp Ile Ile
485 490 495

Lys Gln Lys Thr Gln Ile Pro Leu Gln Ala Arg Glu Leu Ile Asp Thr
500 505 510

Ile Trp Val Lys Gly Asn Ala Ala Ala Asn Ile Phe Lys Asn Cys Leu
515 520 525

Lys Glu Ile Asp Ser Thr Leu Tyr Lys Asn Leu Phe Val Asp Lys Asn
530 535 540

Met Lys Tyr Ile Pro Thr Glu Asp Val Ser Gly Leu Ser Leu Glu Glu
545 550 555 560

Gln Leu Arg Arg Leu Gln Glu Glu Arg Thr Cys Lys Val Cys Met Asp
565 570 575

Lys Glu Val Ser Val Val Phe Ile Pro Cys Gly His Leu Val Val Cys
580 585 590

Gln Glu Cys Ala Pro Ser Leu Arg Lys Cys Pro Ile Cys Arg Gly Ile
595 600 605

Ile Lys Gly Thr Val Arg Thr Phe Leu Ser
610 615

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2100 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GACACTCTGC TGGGCGGCGG GCCGCCCTCC TCCGGGACCT CCCCTCGGGA ACCGTCGCCC 60

GCGGCGCTTA GTTAGGACTG GAGTGCTTGG CGCGAAAAGG TGGACAAGTC CTATTTTCCA 120

GAGAAGATGA CTTTTAACAG TTTTGAAGGA ACTAGAACTT TTGTACTTGC AGACACCAAT 180

AAGGATGAAG AATTTGTAGA AGAGTTTAAT AGATTAAAAA CATTTGCTAA CTTCCCAAGT 240

AGTAGTCCTG TTTCAGCATC AACATTGGCG CGAGCTGGGT TTCTTTATAC CGGTGAAGGA 300

GACACCGTGC AATGTTTCAG TTGTCATGCG GCAATAGATA GATGGCAGTA TGGAGACTCA 360

GCTGTTGGAA GACACAGGAG AATATCCCCA AATTGCAGAT TTATCAATGG TTTTTATTTT 420

GAAAATGGTG CTGCACAGTC TACAAATCCT GGTATCCAAA ATGGCCAGTA CAAATCTGAA 480

AACTGTGTGG GAAATAGAAA TCCTTTTGCC CCTGACAGGC CACCTGAGAC TCATGCTGAT 540

TATCTCTTGA GAACTGGACA GGTTGTAGAT ATTTCAGACA CCATATACCC GAGGAACCCT 600

GCCATGTGTA GTGAAGAAGC CAGATTGAAG TCATTTCAGA ACTGGCCGGA CTATGCTCAT 660

TTAACCCCCA GAGAGTTAGC TAGTGCTGGC CTCTACTACA CAGGGGCTGA TGATCAAGTG 720

CAATGCTTTT GTTGTGGGGG AAAACTGAAA AATTGGGAAC CCTGTGATCG TGCCTGGTCA 780

GAACACAGGA GACACTTTCC CAATTGCTTT TTTGTTTTGG GCCGGAACGT TAATGTTCGA 840

AGTGAATCTG GTGTGAGTTC TGATAGGAAT TTCCCAAATT CAACAAACTC TCCAAGAAAT 900

CCAGCCATGG CAGAATATGA AGCACGGATC GTTACTTTTG GAACATGGAT ATACTCAGTT 960

AACAAGGAGC AGCTTGCAAG AGCTGGATTT TATGCTTTAG GTGAAGGCGA TAAAGTGAAC 1020

TGCTTCCACT GTGGAGGAGG GCTCACGGAT TGGAAGCCAA GTGAAGACCC CTGGGACCAG 1080

CATGCTAAGT GCTACCCAGG GTGCAAATAC CTATTGGATG AGAAGGGGCA AGAATATATA 1140

AATAATATTC ATTTAACCCA TCCACTTGAG GAATCTTTGG GAAGAACTGC TGAAAAAACA 1200

CCACCGCTAA CTAAAAAAAT CGATGATACC ATCTTCCAGA ATCCTATGGT GCAAGAAGCT 1260

ATACGAATGG GATTTAGCTT CAAGGACCTT AAGAAAACAA TGGAAGAAAA AATCCAAACA 1320

TCCGGGAGCA GCTATCTATC ACTTGAGGTC CTGATTGCAG ATCTTGTGAG TGCTCAGAAA 1380

GATAATACGG AGGATGAGTC AAGTCAAACT TCATTGCAGA AAGACATTAG TACTGAAGAG 1440

CAGCTAAGGC GCCTACAAGA GGAGAAGCTT TCCAAAATCT GTATGGATAG AAATATTGCT 1500

ATCGTTTTTT TTCCTTGTGG ACATCTGGCC ACTTGTAAAC AGTGTGCAGA AGCAGTTGAC 1560

AAATGTCCCA TGTGCTACAC CGTCATTACG TTCAACCAAA AAATTTTTAT GTCTTAGTGG 1620

GGCACCACAT GTTATGTTCT TCTTGCTCTA ATTGAATGTG TAATGGGAGC GAACTTTAAG 1680

TAATCCTGCA TTTGCATTCC ATTAGCATCC TGCTGTTTCC AAATGGAGAC CAATGCTAAC 1740

AGCACTGTTT CCGTCTAAAC ATTCAATTTC TGGATCTTTC GAGTTATCAG CTGTATCATT 1800

TAGCCAGTGT TTTACTCGAT TGAAACCTTA GACAGAGAAG CATTTTATAG CTTTTCACAT 1860

GTATATTGGT AGTACACTGA CTTGATTTCT ATATGTAAGT GAATTCATCA CCTGCATGTT 1920

TCATGCCTTT TGCATAAGCT TAACAAATGG AGTGTTCTGT ATAAGCATGG AGATGTGATG 1980

GAATCTGCCC AATGACTTTA ATTGGCTTAT TGTAAACACG GAAAGAACTG CCCCACGCTG 2040

CTGGGAGGAT AAAGATTGTT TTAGATGCTC ACTTCTGTGT TTTAGGATTC TGCCCATTTA 2100

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 496 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Thr Phe Asn Ser Phe Glu Gly Thr Arg Thr Phe Val Leu Ala Asp
1 5 10 15

Thr Asn Lys Asp Glu Glu Phe Val Glu Glu Phe Asn Arg Leu Lys Thr
20 25 30

Phe Ala Asn Phe Pro Ser Ser Ser Pro Val Ser Ala Ser Thr Leu Ala
35 40 45

Arg Ala Gly Phe Leu Tyr Thr Gly Glu Gly Asp Thr Val Gln Cys Phe
50 55 60

Ser Cys His Ala Ala Ile Asp Arg Trp Gln Tyr Gly Asp Ser Ala Val
65 70 75 80

Gly Arg His Arg Arg Ile Ser Pro Asn Cys Arg Phe Ile Asn Gly Phe
85 90 95

Tyr Phe Glu Asn Gly Ala Ala Gln Ser Thr Asn Pro Gly Ile Gln Asn
100 105 110

Gly Gln Tyr Lys Ser Glu Asn Cys Val Gly Asn Arg Asn Pro Phe Ala
115 120 125

Pro Asp Arg Pro Pro Glu Thr His Ala Asp Tyr Leu Leu Arg Thr Gly
130 135 140

Gln Val Val Asp Ile Ser Asp Thr Ile Tyr Pro Arg Asn Pro Ala Met
145 150 155 160

Cys Ser Glu Glu Ala Arg Leu Lys Ser Phe Gln Asn Trp Pro Asp Tyr
165 170 175

Ala His Leu Thr Pro Arg Glu Leu Ala Ser Ala Gly Leu Tyr Tyr Thr
180 185 190

Gly Ala Asp Asp Gln Val Gln Cys Phe Cys Cys Gly Gly Lys Leu Lys
195 200 205

Asn Trp Glu Pro Cys Asp Arg Ala Trp Ser Glu His Arg Arg His Phe
210 215 220

Pro Asn Cys Phe Phe Val Leu Gly Arg Asn Val Asn Val Arg Ser Glu
225 230 235 240

Ser Gly Val Ser Ser Asp Arg Asn Phe Pro Asn Ser Thr Asn Ser Pro
245 250 255

Arg Asn Pro Ala Met Ala Glu Tyr Glu Ala Arg Ile Val Thr Phe Gly
260 265 270

Thr Trp Ile Tyr Ser Val Asn Lys Glu Gln Leu Ala Arg Ala Gly Phe
275 280 285

Tyr Ala Leu Gly Glu Gly Asp Lys Val Lys Cys Phe His Cys Gly Gly
290 295 300

Gly Leu Thr Asp Trp Lys Pro Ser Glu Asp Pro Trp Asp Gln His Ala
305 310 315 320

Lys Cys Tyr Pro Gly Cys Lys Tyr Leu Leu Asp Glu Lys Gly Gln Glu
325 330 335

Tyr Ile Asn Asn Ile His Leu Thr His Pro Leu Glu Glu Ser Leu Gly
340 345 350

Arg Thr Ala Glu Lys Thr Pro Pro Leu Thr Lys Lys Ile Asp Asp Thr
355 360 365

Ile Phe Gln Asn Pro Met Val Gln Glu Ala Ile Arg Met Gly Phe Ser
370 375 380

Phe Lys Asp Leu Lys Lys Thr Met Glu Glu Lys Ile Gln Thr Ser Gly
385 390 395 400

Ser Ser Tyr Leu Ser Leu Glu Val Leu Ile Ala Asp Leu Val Ser Ala
405 410 415

Gln Lys Asp Asn Thr Glu Asp Glu Ser Ser Gln Thr Ser Leu Gln Lys
420 425 430

Asp Ile Ser Thr Glu Glu Gln Leu Arg Arg Leu Gln Glu Glu Lys Leu
435 440 445

Ser Lys Ile Cys Met Asp Arg Asn Ile Ala Ile Val Phe Phe Pro Cys
450 455 460

Gly His Leu Ala Thr Cys Lys Gln Cys Ala Glu Ala Val Asp Lys Cys
465 470 475 480

Pro Met Cys Tyr Thr Val Ile Thr Phe Asn Gln Lys Ile Phe Met Ser
485 490 495

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 67 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Lys Ala Ala Arg Leu Gly Thr Tyr Thr Asn Trp Pro Val Gln Phe Leu
1 5 10 15

Glu Pro Ser Arg Met Ala Ala Ser Gly Phe Tyr Tyr Leu Gly Arg Gly
20 25 30

Asp Glu Val Arg Cys Ala Phe Cys Lys Val Glu Ile Thr Asn Trp Val
35 40 45

Arg Gly Asp Asp Pro Glu Thr Asp His Lys Arg Trp Ala Pro Gln Cys
50 55 60

Pro Phe Val
65

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 275 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Ser Asp Leu Arg Leu Glu Glu Val Arg Leu Asn Thr Phe Glu Lys
1 5 10 15

Trp Pro Val Ser Phe Leu Ser Pro Glu Thr Met Ala Lys Asn Gly Phe
20 25 30

Tyr Tyr Leu Gly Arg Ser Asp Glu Val Arg Cys Ala Phe Cys Lys Val
35 40 45

Glu Ile Met Arg Trp Lys Glu Gly Glu Asp Pro Ala Ala Asp His Lys
50 55 60

Lys Trp Ala Pro Gln Cys Pro Phe Val Lys Gly Ile Asp Val Cys Gly
65 70 75 80

Ser Ile Val Thr Thr Asn Asn Ile Gln Asn Thr Thr Thr His Asp Thr
85 90 95

Ile Ile Gly Pro Ala His Pro Lys Tyr Ala His Glu Ala Ala Arg Val
100 105 110

Lys Ser Phe His Asn Trp Pro Arg Cys Met Lys Gln Arg Pro Glu Gln
115 120 125

Met Ala Asp Ala Gly Phe Phe Tyr Thr Gly Tyr Gly Asp Asn Thr Lys
130 135 140

Cys Phe Tyr Cys Asp Gly Gly Leu Lys Asp Trp Glu Pro Glu Asp Val
145 150 155 160

Pro Trp Glu Gln His Val Arg Trp Phe Asp Arg Cys Ala Tyr Val Gln
165 170 175

Leu Val Lys Gly Arg Asp Tyr Val Gln Lys Val Ile Thr Glu Ala Cys
180 185 190

Val Leu Pro Gly Glu Asn Thr Thr Val Ser Thr Ala Ala Pro Val Ser
195 200 205

Glu Pro Ile Pro Glu Thr Lys Ile Glu Lys Glu Pro Gln Val Glu Asp
210 215 220

Ser Lys Leu Cys Lys Ile Cys Tyr Val Glu Glu Cys Ile Val Cys Phe
225 230 235 240

Val Pro Cys Gly His Val Val Ala Cys Ala Lys Cys Ala Leu Ser Val
245 250 255

Asp Lys Cys Pro Met Cys Arg Lys Ile Val Thr Ser Val Leu Lys Val
260 265 270

Tyr Phe Ser
275

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 498 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met Thr Glu Leu Gly Met Glu Leu Glu Ser Val Arg Leu Ala Thr Phe
1 5 10 15

Gly Glu Trp Pro Leu Asn Ala Pro Val Ser Ala Glu Asp Leu Val Ala
20 25 30

Asn Gly Phe Phe Ala Thr Gly Lys Trp Leu Glu Ala Glu Cys His Phe
35 40 45

Cys His Val Arg Ile Asp Arg Trp Glu Tyr Gly Asp Gln Val Ala Glu
50 55 60

Arg His Arg Arg Ser Ser Pro Ile Cys Ser Met Val Leu Ala Pro Asn
65 70 75 80

His Cys Gly Asn Val Pro Arg Ser Gln Glu Ser Asp Asn Glu Gly Asn
85 90 95

Ser Val Val Asp Ser Pro Glu Ser Cys Ser Cys Pro Asp Leu Leu Leu
100 105 110

Glu Ala Asn Arg Leu Val Thr Phe Lys Asp Trp Pro Asn Pro Asn Ile
115 120 125

Thr Pro Gln Ala Leu Ala Lys Ala Gly Phe Tyr Tyr Leu Asn Arg Leu
130 135 140

Asp His Val Lys Cys Val Trp Cys Asn Gly Val Ile Ala Lys Trp Glu
145 150 155 160

Lys Asn Asp Asn Ala Phe Glu Glu His Lys Arg Phe Phe Pro Gln Cys
165 170 175

Pro Arg Val Gln Met Gly Pro Leu Ile Glu Phe Ala Thr Gly Lys Asn
180 185 190

Leu Asp Glu Leu Gly Ile Gln Pro Thr Thr Leu Pro Leu Arg Pro Lys
195 200 205

Tyr Ala Cys Val Asp Ala Arg Leu Arg Thr Phe Thr Asp Trp Pro Ile
210 215 220

Ser Asn Ile Gln Pro Ala Ser Ala Leu Ala Gln Ala Gly Leu Tyr Tyr
225 230 235 240

Gln Lys Ile Gly Asp Gln Val Arg Cys Phe His Cys Asn Ile Gly Leu
245 250 255

Arg Ser Trp Gln Lys Glu Asp Glu Pro Trp Phe Glu His Ala Lys Trp
260 265 270

Ser Pro Lys Cys Gln Phe Val Leu Leu Ala Lys Gly Pro Ala Tyr Val
275 280 285

Ser Glu Val Leu Ala Thr Thr Ala Ala Asn Ala Ser Ser Gln Pro Ala
290 295 300

Thr Ala Pro Ala Pro Thr Leu Gln Ala Asp Val Leu Met Asp Glu Ala
305 310 315 320

Pro Ala Lys Glu Ala Leu Thr Leu Gly Ile Asp Gly Gly Val Val Arg
325 330 335

Asn Ala Ile Gln Arg Lys Leu Leu Ser Ser Gly Cys Ala Phe Ser Thr
340 345 350

Leu Asp Glu Leu Leu His Asp Ile Phe Asp Asp Ala Gly Ala Gly Ala
355 360 365

Ala Leu Glu Val Arg Glu Pro Pro Glu Pro Ser Ala Pro Phe Ile Glu
370 375 380

Pro Cys Gln Ala Thr Thr Ser Lys Ala Ala Ser Val Pro Ile Pro Val
385 390 395 400

Ala Asp Ser Ile Pro Ala Lys Pro Gln Ala Ala Glu Ala Val Ser Asn
405 410 415

Ile Ser Lys Ile Thr Asp Glu Ile Gln Lys Met Ser Val Ser Thr Pro
420 425 430

Asn Gly Asn Leu Ser Leu Glu Glu Glu Asn Arg Gln Leu Lys Asp Ala
435 440 445

Arg Leu Cys Lys Val Cys Leu Asp Glu Glu Val Gly Val Val Phe Leu
450 455 460

Pro Cys Gly His Leu Ala Thr Cys Asn Gln Cys Ala Pro Ser Val Ala
465 470 475 480

Asn Cys Pro Met Cys Arg Ala Asp Ile Lys Gly Phe Val Arg Thr Phe
485 490 495

Leu Ser

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 67 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Glu Glu Val Arg Leu Asn Thr Phe Glu Lys Trp Pro Val Ser Phe Leu
1 5 10 15

Ser Pro Glu Thr Met Ala Lys Asn Gly Phe Tyr Tyr Leu Gly Arg Ser
20 25 30

Asp Glu Val Arg Cys Ala Phe Cys Lys Val Glu Ile Met Arg Trp Lys
35 40 45

Glu Gly Glu Asp Pro Ala Ala Asp His Lys Lys Trp Ala Pro Gln Cys
50 55 60

Pro Phe Val
65

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 67 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Glu Ala Asn Arg Leu Val Thr Phe Lys Asp Trp Pro Asn Pro Asn Ile
1 5 10 15

Thr Pro Gln Ala Leu Ala Lys Ala Gly Phe Tyr Tyr Leu Asn Arg Leu
20 25 30

Asp His Val Lys Cys Val Trp Cys Asn Gly Val Ile Ala Lys Trp Glu
35 40 45

Lys Asn Asp Asn Ala Phe Glu Glu His Lys Arg Phe Phe Pro Gln Cys
50 55 60

Pro Arg Val
65

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Glu Phe Asn Arg Leu Lys Thr Phe Ala Asn Phe Pro Ser Ser Ser Pro
1 5 10 15

Val Ser Ala Ser Thr Leu Ala Arg Ala Gly Phe Leu Tyr Thr Gly Glu
20 25 30

Gly Asp Thr Val Gln Cys Phe Ser Cys His Ala Ala Ile Asp Arg Trp
35 40 45

Gln Tyr Gly Asp Ser Ala Val Gly Arg His Arg Arg Ile Ser Pro Asn
50 55 60

Cys Arg Phe Ile
65

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Glu Phe Asn Arg Leu Lys Thr Phe Ala Asn Phe Pro Ser Gly Ser Pro
1 5 10 15

Val Ser Ala Ser Thr Leu Ala Arg Ala Gly Phe Leu Tyr Thr Gly Glu
20 25 30

Gly Asp Thr Val Arg Cys Phe Ser Cys His Ala Ala Val Asp Arg Trp
35 40 45

Gln Tyr Gly Asp Ser Ala Val Gly Arg His Arg Lys Val Ser Pro Asn
50 55 60

Cys Arg Phe Ile
65

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Glu Leu Tyr Arg Met Ser Thr Tyr Ser Thr Phe Pro Ala Gly Val Pro
1 5 10 15

Val Ser Glu Arg Ser Leu Ala Arg Ala Gly Phe Tyr Tyr Thr Gly Val
20 25 30

Asn Asp Lys Val Lys Cys Phe Cys Cys Gly Leu Met Leu Asp Asn Trp
35 40 45

Lys Arg Gly Asp Ser Pro Thr Glu Lys His Lys Lys Leu Tyr Pro Ser
50 55 60

Cys Arg Phe Val
65

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Glu Leu Tyr Arg Met Ser Thr Tyr Ser Thr Phe Pro Ala Gly Val Pro
1 5 10 15

Val Ser Glu Arg Ser Leu Ala Arg Ala Gly Phe Tyr Tyr Thr Gly Val
20 25 30

Asn Asp Lys Val Lys Cys Phe Cys Cys Gly Leu Met Leu Asp Asn Trp
35 40 45

Lys Leu Gly Asp Ser Pro Ile Gln Lys His Lys Gln Leu Tyr Pro Ser
50 55 60

Cys Ser Phe Ile
65

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Glu Glu Ala Arg Leu Lys Ser Phe Gln Asn Trp Pro Asp Tyr Ala His
1 5 10 15

Leu Thr Pro Arg Glu Leu Ala Ser Ala Gly Leu Tyr Tyr Thr Gly Ala
20 25 30

Asp Asp Gln Val Gln Cys Phe Cys Cys Gly Gly Lys Leu Lys Asn Trp
35 40 45

Glu Pro Cys Asp Arg Ala Trp Ser Glu His Arg Arg His Phe Pro Asn
50 55 60

Cys Phe Phe Val
65

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Glu Glu Ala Arg Leu Lys Ser Phe Gln Asn Trp Pro Asp Tyr Ala His
1 5 10 15

Leu Thr Pro Arg Glu Leu Ala Ser Ala Gly Leu Tyr Tyr Thr Gly Ile
20 25 30

Gly Asp Gln Val Gln Cys Phe Cys Cys Gly Gly Lys Leu Lys Asn Trp
35 40 45

Glu Pro Cys Asp Arg Ala Trp Ser Glu His Arg Arg His Phe Pro Asn
50 55 60

Cys Phe Phe Val
65

(2). INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 67 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Glu Asn Ala Arg Leu Leu Thr Phe Gln Thr Trp Pro Leu Thr Phe Leu
1 5 10 15

Ser Pro Thr Asp Leu Ala Arg Ala Gly Phe Tyr Tyr Ile Gly Pro Gly
20 25 30

Asp Arg Val Ala Cys Phe Ala Cys Gly Gly Lys Leu Ser Asn Trp Glu
35 40 45

Pro Lys Asp Asn Ala Met Ser Glu His Leu Arg His Phe Pro Lys Cys
50 55 60

Pro Phe Ile
65

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 67 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Glu Glu Ala Arg Phe Leu Thr Tyr His Met Trp Pro Leu Thr Phe Leu
1 5 10 15

Ser Pro Ser Glu Leu Ala Arg Ala Gly Phe Tyr Tyr Ile Gly Pro Gly
20 25 30

Asp Arg Val Ala Cys Phe Ala Cys Gly Gly Lys Leu Ser Asn Trp Glu
35 40 45

Pro Lys Asp Asp Ala Met Ser Glu His Arg Arg His Phe Pro Asn Cys
50 55 60

Pro Phe Leu
65

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Tyr Glu Ala Arg Ile Val Thr Phe Gly Thr Trp Ile Tyr Ser Val Asn
1 5 10 15

Lys Glu Gln Leu Ala Arg Ala Gly Phe Tyr Ala Leu Gly Glu Gly Asp
20 25 30

Lys Val Lys Cys Phe His Cys Gly Gly Gly Leu Thr Asp Trp Lys Pro
35 40 45

Ser Glu Asp Pro Trp Asp Gln His Ala Lys Cys Tyr Pro Gly Cys Lys
50 55 60

Tyr Leu
65

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Tyr Glu Ala Arg Ile Phe Thr Phe Gly Thr Trp Ile Tyr Ser Val Asn
1 5 10 15

Lys Glu Gln Leu Ala Arg Ala Gly Phe Tyr Ala Leu Gly Glu Gly Asp
20 25 30

Lys Val Lys Cys Phe His Cys Gly Gly Gly Leu Thr Asp Trp Lys Pro
35 40 45

Ser Glu Asp Pro Trp Glu Gln His Ala Lys Trp Tyr Pro Gly Cys Lys
50 55 60

Tyr Leu
65

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

His Ala Ala Arg Phe Lys Thr Phe Phe Asn Trp Pro Ser Ser Val Leu
1 5 10 15

Val Asn Pro Glu Gln Leu Ala Ser Ala Gly Phe Tyr Tyr Val Gly Asn
20 25 30

Ser Asp Asp Val Lys Cys Phe Cys Cys Asp Gly Gly Leu Arg Cys Trp
35 40 45

Glu Ser Gly Asp Asp Pro Trp Val Gln His Ala Lys Trp Phe Pro Arg
50 55 60

Cys Glu Tyr Leu
65

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

His Ala Ala Arg Met Arg Thr Phe Met Tyr Trp Pro Ser Ser Val Pro
1 5 10 15

Val Gln Pro Glu Gln Leu Ala Ser Ala Gly Phe Tyr Tyr Val Gly Arg
20 25 30

Asn Asp Asp Val Lys Cys Phe Gly Cys Asp Gly Gly Leu Arg Cys Trp
35 40 45

Glu Ser Gly Asp Asp Pro Trp Val Glu His Ala Lys Trp Phe Pro Arg
50 55 60

Cys Glu Phe Leu
65

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Glu Ala Ala Arg Leu Arg Thr Phe Ala Glu Trp Pro Arg Gly Leu Lys
1 5 10 15

Gln Arg Pro Glu Glu Leu Ala Glu Ala Gly Phe Phe Tyr Thr Gly Gln
20 25 30

Gly Asp Lys Thr Arg Cys Phe Cys Cys Asp Gly Gly Leu Lys Asp Trp
35 40 45

Glu Pro Asp Asp Ala Pro Trp Gln Gln His Ala Arg Trp Tyr Asp Arg
50 55 60

Cys Glu Tyr Val
65

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Glu Ala Ala Arg Val Lys Ser Phe His Asn Trp Pro Arg Cys Met Lys
1 5 10 15

Gln Arg Pro Glu Gln Met Ala Asp Ala Gly Phe Phe Tyr Thr Gly Tyr
20 25 30

Gly Asp Asn Thr Lys Cys Phe Tyr Cys Asp Gly Gly Leu Lys Asp Trp
35 40 45

Glu Pro Glu Asp Val Pro Trp Glu Gln His Val Arg Trp Phe Asp Arg
50 55 60

Cys Ala Tyr Val
65

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 68 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```
Val Asp Ala Arg Leu Arg Thr Phe Thr Asp Trp Pro Ile Ser Asn Ile
1               5                  10                  15

Gln Pro Ala Ser Ala Leu Ala Gln Ala Gly Leu Tyr Tyr Gln Lys Ile
            20                  25                  30

Gly Asp Gln Val Arg Cys Phe His Cys Asn Ile Gly Leu Arg Ser Trp
        35                  40                  45

Gln Lys Glu Asp Glu Pro Trp Phe Glu His Ala Lys Trp Ser Pro Lys
    50                  55                  60

Cys Gln Phe Val
65
```

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```
Glu Ser Val Arg Leu Ala Thr Phe Gly Glu Trp Pro Leu Asn Ala Pro
1               5                  10                  15

Val Ser Ala Glu Asp Leu Val Ala Asn Gly Phe Phe Gly Thr Trp Met
            20                  25                  30

Glu Ala Glu Cys Asp Phe Cys His Val Arg Ile Asp Arg Trp Glu Tyr
        35                  40                  45

Gly Asp Leu Val Ala Glu Arg His Arg Arg Ser Ser Pro Ile Cys Ser
    50                  55                  60

Met Val
65
```

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant

(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Glu Gln Leu Arg Arg Leu Gln Glu Glu Arg Thr Cys Lys Val Cys Met
1 5 10 15

Asp Lys Glu Val Ser Val Val Phe Ile Pro Cys Gly His Leu Val Val
20 25 30

Cys Gln Glu Cys Ala Pro Ser Leu Arg Lys Cys Pro Ile Cys
35 40 45

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Glu Gln Leu Arg Arg Leu Pro Glu Glu Arg Thr Cys Lys Val Cys Met
1 5 10 15

Asp Lys Glu Val Ser Ile Val Phe Ile Pro Cys Gly His Leu Val Val
20 25 30

Cys Lys Asp Cys Ala Pro Ser Leu Arg Lys Cys Pro Ile Cys
35 40 45

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Glu Gln Leu Arg Arg Leu Gln Glu Glu Lys Leu Ser Lys Ile Cys Met
1 5 10 15

Asp Arg Asn Ile Ala Ile Val Phe Phe Pro Cys Gly His Leu Ala Thr
20 25 30

Cys Lys Gln Cys Ala Glu Ala Val Asp Lys Cys Pro Met Cys
35 40 45

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Glu Gln Leu Arg Arg Leu Gln Glu Glu Lys Leu Cys Lys Ile Cys Met
1 5 10 15

Asp Arg Asn Ile Ala Ile Val Phe Val Pro Cys Gly His Leu Val Thr
20 25 30

Cys Lys Gln Cys Ala Glu Ala Val Asp Lys Cys Pro Met Cys
35 40 45

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Glu Glu Asn Arg Gln Leu Lys Asp Ala Arg Leu Cys Lys Val Cys Leu
1 5 10 15

Asp Glu Glu Val Gly Val Val Phe Leu Pro Cys Gly His Leu Ala Thr
20 25 30

Cys Asn Gln Cys Ala Pro Ser Val Ala Asn Cys Pro Met Cys
35 40 45

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Glu Lys Glu Pro Gln Val Glu Asp Ser Lys Leu Cys Lys Ile Cys Tyr
1 5 10 15

Val Glu Glu Cys Ile Val Cys Phe Val Pro Cys Gly His Val Val Ala
20 25 30

Cys Ala Lys Cys Ala Leu Ser Val Asp Lys Cys Pro Met Cys
35 40 45

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Ala Val Glu Ala Glu Val Ala Asp Asp Arg Leu Cys Lys Ile Cys Leu
1 5 10 15

Gly Ala Glu Lys Thr Val Cys Phe Val Pro Cys Gly His Val Val Ala
20 25 30

Cys Gly Lys Cys Ala Ala Gly Val Thr Thr Cys Pro Val Cys
35 40 45

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2474 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GAATTCCGGG AGACCTACAC CCCCGGAGAT CAGAGGTCAT TGCTGGCGTT CAGAGCCTAG 60

GAAGTGGGCT GCGGTATCAG CCTAGCAGTA AAACCGACCA GAAGCCATGC 120

ATCCCCAGAG AAAGACTTGT CCCTTCCCCT CCCTGTCATC TCACCATGAA CATGGTTCAA 180

GACAGCGCCT TTCTAGCCAA GCTGATGAAG AGTGCTGACA CCTTTGAGTT GAAGTATGAC 240

TTTTCCTGTG AGCTGTACCG ATTGTCCACG TATTCAGCTT TTCCCAGGGG AGTTCCTGTG 300

TCAGAAAGGA GTCTGGCTCG TGCTGGCTTT TACTACACTG GTGCCAATGA CAAGGTCAAG 360

TGCTTCTGCT GTGGCCTGAT GCTAGACAAC TGGAAACAAG GGGACAGTCC CATGGAGAAG 420

CACAGAAAGT TGTACCCCAG CTGCAACTTT GTACAGACTT TGAATCCAGC CAACAGTCTG 480

GAAGCTAGTC CTCGGCCTTC TCTTCCTTCC ACGGCGATGA GCACCATGCC TTTGAGCTTT 540

GCAAGTTCTG AGAATACTGG CTATTTCAGT GGCTCTTACT CGAGCTTTCC CTCAGACCCT 600

GTGAACTTCC GAGCAAATCA AGATTGTCCT GCTTTGAGCA CAAGTCCCTA CCACTTTGCA 660

ATGAACACAG AGAAGGCCAG ATTACTCACC TATGAAACAT GGCCATTGTC TTTTCTGTCA 720

CCAGCAAAGC TGGCCAAAGC AGGCTTCTAC TACATAGGAC CTGGAGATAG AGTGGCCTGC 780

TTTGCGTGCG ATGGGAAACT GAGCAACTGG GAACGTAAGG ATGATGCTAT GTCAGAGCAC 840

CAGAGGCATT TCCCCAGCTG TCCGTTCTTA AAAGACTTGG GTCAGTCTGC TTCGAGATAC 900

ACTGTCTCTA ACCTGAGCAT GCAGACACAC GCAGCCCGTA TTAGAACATT CTCTAACTGG 960

CCTTCTAGTG CACTAGTTCA TTCCCAGGAA CTTGCAAGTG CGGGCTTTTA TTATACAGCA 1020

CACAGTGATG ATGTCAAGTG TTTATGCTGT GATGGTGGGC TGAGGTGCTG GGAATCTGGA 1080

GATGACCCCT GGGTGGAACA TGCCAAGTGG TTTCCAAGGT GTGAGTACTT GCTCAGAATC 1140

AAAGGCCAAG AATTTGTCAG CCAAGTTCAA GCTGGCTATC CTCATCTACT TGAGCAGCTA 1200

TTATCTACGT CAGACTCCCC AGAAGATGAG AATGCAGACG CAGCAATCGT GCATTTTGGC 1260

CCTGGAGAAA GTTCGGAAGA TGTCGTCATG ATGAGCACGC CTGTGGTTAA AGCAGCCTTG 1320

GAAATGGGCT TCAGTAGGAG CCTGGTGAGA CAGACGGTTC AGTGGCAGAT CCTGGCCACT 1380

GGTGAGAACT ACAGGACCGT CAGTGACCTC GTTATAGGCT TACTCGATGC AGAAGACGAG 1440

ATGAGAGAGG AGCAGATGGA GCAGGCGGCC CAGGAGGAGG AGTCAGATGA TCTAGCACTA 1500

ATCCGGAAGA ACAAAATGGT GCTTTTCCAA CATTTGACGT GTGTGACACC AATGCTGTAT 1560

TGCCTCCTAA GTGCAAGGGC CATCACTGAA CAGGAGTGCA ATGCTGTGAA ACAGAAACCA 1620

CACACCTTAC AAGCAAGCAC ACTGATTGAT ACTGTGTTAG CAAAAGGAAA CACTGCAGCA 1680

ACCTCATTCA GAAACTCCCT TCGGGAAATT GACCCTGCGT TATACAGAGA TATATTTGTG 1740

CAACAGGACA TTAGGAGTCT TCCCACACAT GACATTGCAG CTCTACCAAT GGAAGAACAG 1800

TTGCGGCCCC TCCCGGAGGA CAGAATGTGT AAAGTGTGTA TGGACCGAGA GGTATCCATC 1860

GTGTTCATTC CCTGTGGCCA TCTGGTCGTG TGCAAAGACT GCGCTCCCTC TCTGAGGAAG 1920

TGTCCCATCT GTAGAGGGAC CATCAAGGGC ACAGTGCGCA CATTTCTCTC CTGAACAAGA 1980

CTAATGGTCC ATGGCTGCAA CTTCAGCCAG GAGGAAGTTC ACTGTCACTC CCAGTTCCAT 2040

TCGGAACTTG AGGCCAGCCT GGATAGCACG AGACACCGCC AAACACACAA ATATAAACAT 2100

GAAAAACTTT TGTCTGAAGT CAAGAATGAA TGAATTACTT ATATAATAAT TTTAATTGGT 2160

TTCCTTAAAA GTGCTATTTG TTCCCAACTC AGAAAATTGT TTTCTGTAAA CATATTTACA 2220

TACTACCTGC ATCTAAAGTA TTCATATATT CATATATTCA GATGTCATGA GAGAGGGTTT 2280

TGTTCTTGTT CCTGAAAAGC TGGTTTATCA TCTGATCAGC ATATACTGCG CAACGGGCAG 2340

GGCTAGAATC CATGAACCAA GCTGCAAAGA TCTCACGCTA AATAAGGCGG AAAGATTTGG 2400

AGAAACGAAA GGAAATTCTT TCCTGTCCAA TGTATACTCT TCAGACTAAT 2460

TATCAAGCCT 2474

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 602 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Met Asn Met Val Gln Asp Ser Ala Phe Leu Ala Lys Leu Met Lys Ser
1 5 10 15

Ala Asp Thr Phe Glu Leu Lys Tyr Asp Phe Ser Cys Glu Leu Tyr Arg
20 25 30

Leu Ser Thr Tyr Ser Ala Phe Pro Arg Gly Val Pro Val Ser Glu Arg
35 40 45

Ser Leu Ala Arg Ala Gly Phe Tyr Tyr Thr Gly Ala Asn Asp Lys Val
50 55 60

Lys Cys Phe Cys Cys Gly Leu Met Leu Asp Asn Trp Lys Gln Gly Asp
65 70 75 80

Ser Pro Met Glu Lys His Arg Lys Leu Tyr Pro Ser Cys Asn Phe Val
85 90 95

Gln Thr Leu Asn Pro Ala Asn Ser Leu Glu Ala Ser Pro Arg Pro Ser
100 105 110

Leu Pro Ser Thr Ala Met Ser Thr Met Pro Leu Ser Phe Ala Ser Ser
115 120 125

Glu Asn Thr Gly Tyr Phe Ser Gly Ser Tyr Ser Ser Phe Pro Ser Asp
130 135 140

Pro Val Asn Phe Arg Ala Asn Gln Asp Cys Pro Ala Leu Ser Thr Ser
145 150 155 160

Pro Tyr His Phe Ala Met Asn Thr Glu Lys Ala Arg Leu Leu Thr Tyr
165 170 175

Glu Thr Trp Pro Leu Ser Phe Leu Ser Pro Ala Lys Leu Ala Lys Ala
180 185 190

Gly Phe Tyr Tyr Ile Gly Pro Gly Asp Arg Val Ala Cys Phe Ala Cys
195 200 205

Asp Gly Lys Leu Ser Asn Trp Glu Arg Lys Asp Asp Ala Met Ser Glu
210 215 220

His Gln Arg His Phe Pro Ser Cys Pro Phe Leu Lys Asp Leu Gly Gln
225 230 235 240

Ser Ala Ser Arg Tyr Thr Val Ser Asn Leu Ser Met Gln Thr His Ala
245 250 255

Ala Arg Ile Arg Thr Phe Ser Asn Trp Pro Ser Ser Ala Leu Val His
260 265 270

Ser Gln Glu Leu Ala Ser Ala Gly Phe Tyr Tyr Thr Gly His Ser Asp
275 280 285

Asp Val Lys Cys Leu Cys Cys Asp Gly Gly Leu Arg Cys Trp Glu Ser
290 295 300

Gly Asp Asp Pro Trp Val Glu His Ala Lys Trp Phe Pro Arg Cys Glu
305 310 315 320

Tyr Leu Leu Arg Ile Lys Gly Gln Glu Phe Val Ser Gln Val Gln Ala
325 330 335

Gly Tyr Pro His Leu Leu Glu Gln Leu Leu Ser Thr Ser Asp Ser Pro
340 345 350

Glu Asp Glu Asn Ala Asp Ala Ala Ile Val His Phe Gly Pro Gly Glu
355 360 365

Ser Ser Glu Asp Val Val Met Met Ser Thr Pro Val Val Lys Ala Ala
370 375 380

Leu Glu Met Gly Phe Ser Arg Ser Leu Val Arg Gln Thr Val Gln Trp
385 390 395 400

Gln Ile Leu Ala Thr Gly Glu Asn Tyr Arg Thr Val Ser Asp Leu Val
405 410 415

Ile Gly Leu Leu Asp Ala Glu Asp Glu Met Arg Glu Glu Gln Met Glu
420 425 430

Gln Ala Ala Glu Glu Glu Glu Ser Asp Asp Leu Ala Leu Ile Arg Lys
435 440 445

Asn Lys Met Val Leu Phe Gln His Leu Thr Cys Val Thr Pro Met Leu
450 455 460

Tyr Cys Leu Leu Ser Ala Arg Ala Ile Thr Glu Gln Glu Cys Asn Ala
465 470 475 480

Val Lys Gln Lys Pro His Thr Leu Gln Ala Ser Thr Leu Ile Asp Thr
485 490 495

Val Leu Ala Lys Gly Asn Thr Ala Ala Thr Ser Phe Arg Asn Ser Leu
500 505 510

Arg Glu Ile Asp Pro Ala Leu Tyr Arg Asp Ile Phe Val Gln Gln Asp
515 520 525

Ile Arg Ser Leu Pro Thr Asp Asp Ile Ala Ala Leu Pro Met Glu Glu
530 535 540

Gln Leu Arg Pro Leu Pro Glu Asp Arg Met Cys Lys Val Cys Met Asp
545 550 555 560

Arg Glu Val Ser Ile Val Phe Ile Pro Cys Gly His Leu Val Val Cys
565 570 575

Lys Asp Cys Ala Pro Ser Leu Arg Lys Cys Pro Ile Cys Arg Gly Thr
580 585 590

Ile Lys Gly Thr Val Arg Thr Phe Leu Ser
595 600

(2). INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2416 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

CTGTGGTGGA GATCTATTGT CCAAGTGGTG AGAAACTTCA TCTGGAAGTT TAAGCGGTCA 60

GAAATACTAT TACTACTCAT GGACAAAACT GTCTCCCAGA GACTCGCCCA AGGTACCTTA 120

CACCCAAAAA CTTAAACGTA TAATGGAGAA CAGCACAATC TTGTCAAATT GGACAAAGGA 180

GAGCGAAGAA AAAATGAAGT TTGACTTTTC GTGTGAACTC TACCGAATGT CTACATATTC 240

AGCTTTTCCC AGGGGAGTTC CTGTCTCAGA GAGGAGTCTG GCTCGTGCTG GCTTTTATTA 300

TACAGGTGTG AATGACAAAG TCAAGTGCTT CTGCTGTGGC CTGATGTTGG ATAACTGGAA 360

ACAAGGGGAC AGTCCTGTTG AAAAGCACAG ACAGTTCTAT CCCAGCTGCA GCTTTGTACA 420

GACTCTGCTT TCAGCCAGTC TGCAGTCTCC ATCTAAGAAT ATGTCTCCTG TGAAAAGTAG 480

ATTTGCACAT TCGTCACCTC TGGAACGAGG TGGCATTCAC TCCAACCTGT GCTCTAGCCC 540

TCTTAATTCT AGAGCAGTGG AAGACTTCTC ATCAAGGATG GATCCCTGCA GCTATGCCAT 600

GAGTACAGAA GAGGCCAGAT TTCTTACTTA CAGTATGTGG CCTTTAAGTT TTCTGTCACC 660

AGCAGAGCTG GCCAGAGCTG GCTTCTATTA CATAGGGCCT GGAGACAGGG TGGCCTGTTT 720

TGCCTGTGGT GGGAAACTGA GCAACTGGGA ACCAAAGGAT TATGCTATGT CAGAGCACCG 780

CAGACATTTT CCCCACTGTC CATTTCTGGA AAATACTTCA GAAACACAGA GGTTTAGTAT 840

ATCAAATCTA AGTATGCAGA CACACTCTGC TCGATTGAGG ACATTTCTGT ACTGGCCACC 900

TAGTGTTCCT GTTCAGCCCG AGCAGCTTGC AAGTGCTGGA TTCTATTACG TGGATCGCAA 960

TGATGATGTC AAGTGCCTTT GTTGTGATGG TGGCTTGAGA TGTTGGGAAC CTGGAGATGA 1020

CCCCTGGATA GAACACGCCA AATGGTTTCC AAGGTGTGAG TTCTTGATAC GGATGAAGGG 1080

TCACGAGTTT GTTGATGAGA TTCAAGCTAG ATATCCTCAT CTTCTTGAGC AGCTGTTGTC 1140

CACTTCAGAC ACCCCAGGAG AAGAAAATGC TGACCCTACA GAGACAGTGG TGCATTTTGG 1200

CCCTGGAGAA AGTTCGAAAG ATGTCGTCAT GATGAGCACG CCTGTGGTTA AAGCAGCCTT 1260

GGAAATGGGC TTCAGTAGGA GCCTGGTGAG ACAGACGGTT CAGCGGCAGA TCCTGGCCAC 1320

TGGTGAGAAC TACAGGACCG TCAATGATAT TGTCTCAGTA CTTTTGAATG CTGAAGATGA 1380

GAGAAGAGAA GAGGAGAAGG AAAGACAGAC TGAAGAGATG GCATCAGGTG ACTTATCACT 1440

GATTCGGAAG AATAGAATGG CCCTCTTTCA ACAGTTGACA CATGTCCTTC CTATCCTGGA 1500

TAATCTTCTT GAGGCCAGTG TAATTACAAA ACAGGAACAT GATATTATTA GACAGAAAAC 1560

ACAGATACCC TTACAAGCAA GAGAGCTTAT TGACACCGTT TTAGTCAAGG GAAATGCTGC 1620

AGCCAACATC TTCAAAAACT CTCTGAAGGG AATTGACTCC ACGTTATATG AAAACTTATT 1680

TGTGGAAAAG AATATGAAGT ATATTCCAAC AGAAGACGTT TCAGGCTTGT CATTGGAAGA 1740

GCAGTTGCGG AGATTACAAG AAGAACGAAC TTGCAAAGTG TGTATGGACA GAGAGGTTTC 1800

TATTGTGTTC ATTCCGTGTG GTCATCTAGT AGTCTGCCAG GAATGTGCCC CTTCTCTAAG 1860

GAAGTGCCCC ATCTGCAGGG GGACAATCAA GGGGACTGTG CGCACATTTC TCTCATGAGT 1920

GAAGAATGGT CTGAAAGTAT TGTTGGACAT CAGAAGCTGT CAGAACAAAG AATGAACTAC 1980

TGATTTCAGC TCTTCAGCAG GACATTCTAC TCTCTTTCAA GATTAGTAAT CTTGCTTTAT 2040

GAAGGGTAGC ATTGTATATT TAAGCTTAGT CTGTTGCAAG GGAAGGTCTA TGCTGTTGAG 2100

CTACAGGACT GTGTCTGTTC CAGAGCAGGA GTTGGGATGC TTGCTGTATG TCCTTCAGGA 2160

CTTCTTGGGA TTTGGGAATT TGGGGAAAGC TTTGGAATCC AGTGATGTGG AGCTCAGAAA 2220

TCCTGGAACC AGTGACTCTG GTACTCAGTA GATAGGGTAC CCTGTACTTC TTGGTGCTTT 2280

TCCAGTCTGG GAAATAAGGA GGAATCTGCT GCTGGTAAAA ATTTGCTGGA TGTGAGAAAT 2340

AGATGAAAGT GTTTCGGGTG GGGCCGTGCA TCAGTGTAGT GTGTGCAGGG ATGTATGCAG 2400

GCCAAACACT GTGTAG 2416

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 591 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Met Glu Lys Ser Thr Ile Leu Se- Asn Trp Thr Lys Glu Ser Glu Glu
1 5 10 15

Lys Met Lys Phe Asp Phe Ser Cys Glu Leu Tyr Arg Met Ser Thr Tyr
20 25 30

Ser Ala Phe Pro Arg Gly Val Pro Val Ser Glu Arg Ser Leu Ala Arg
35 40 45

Ala Gly Phe Tyr Tyr Thr Gly Val Asn Asp Lys Val Lys Cys Phe Cys
50 55 60

Cys Gly Leu Met Leu Asp Asn Trp Lys Gln Gly Asp Ser Pro Val Glu
65 70 75 80

Lys His Arg Gln Phe Tyr Pro Ser Cys Ser Phe Val Gln Thr Leu Leu
85 90 95

Ser Ala Ser Leu Gln Ser Pro Ser Lys Asn Met Ser Pro Val Lys Ser
100 105 110

Arg Phe Ala His Ser Ser Pro Leu Glu Arg Gly Gly Ile His Ser Asn
115 120 125

Leu Cys Ser Ser Pro Leu Asn Ser Arg Ala Val Glu Asp Phe Ser Ser
130 135 140

Arg Met Asp Pro Cys Ser Tyr Ala Met Ser Thr Glu Glu Ala Arg Phe
145 150 155 160

Leu Thr Tyr Ser Met Trp Pro Leu Ser Phe Leu Ser Pro Ala Glu Leu
165 170 175

Ala Arg Ala Gly Phe Tyr Tyr Ile Gly Pro Gly Asp Arg Val Ala Cys
180 185 190

Phe Ala Cys Gly Gly Lys Leu Ser Asn Trp Glu Pro Lys Asp Tyr Ala
195 200 205

Met Ser Glu His Arg Arg His Phe Pro His Cys Pro Phe Leu Glu Asn
210 215 220

Thr Ser Glu Thr Gln Arg Phe Ser Ile Ser Asn Leu Ser Met Gln Thr
225 230 235 240

His Ser Ala Arg Leu Arg Thr Phe Leu Tyr Trp Pro Pro Ser Val Pro
245 250 255

Val Gln Pro Glu Gln Leu Ala Ser Ala Gly Phe Tyr Tyr Val Asp Arg
260 265 270

Asn Asp Asp Val Lys Cys Leu Cys Cys Asp Gly Gly Leu Arg Cys Trp
275 280 285

Glu Pro Gly Asp Asp Pro Trp Ile Glu His Ala Lys Trp Phe Pro Arg
290 295 300

Cys Glu Phe Leu Ile Arg Met Lys Gly Gln Glu Phe Val Asp Glu Ile
305 310 315 320

Gln Ala Arg Tyr Pro His Leu Leu Glu Gln Leu Leu Ser Thr Ser Asp
325 330 335

Thr Pro Gly Glu Glu Asn Ala Asp Pro Thr Glu Thr Val Val His Phe
340 345 350

Gly Pro Gly Glu Ser Ser Lys Asp Val Val Met Met Ser Thr Pro Val
355 360 365

Val Lys Ala Ala Leu Glu Met Gly Phe Ser Arg Ser Leu Val Arg Gln
370 375 380

Thr Val Gln Arg Gln Ile Leu Ala Thr Gly Glu Asn Tyr Arg Thr Val
385 390 395 400

Asn Asp Ile Val Ser Val Leu Leu Asn Ala Glu Asp Glu Arg Arg Glu
405 410 415

Glu Glu Lys Glu Arg Gln Thr Glu Glu Met Ala Ser Gly Asp Leu Ser
420 425 430

Leu Ile Arg Lys Asn Arg Met Ala Leu Phe Gln Gln Leu Thr His Val
435 440 445

Leu Pro Ile Leu Asp Asn Leu Leu Glu Ala Ser Val Ile Thr Lys Gln
450 455 460

Glu His Asp Ile Ile Arg Gln Lys Thr Gln Ile Pro Leu Gln Ala Arg
465 470 475 480

Glu Leu Ile Asp Thr Val Leu Val Lys Gly Asn Ala Ala Ala Asn Ile
485 490 495

Phe Lys Asn Ser Leu Lys Gly Ile Asp Ser Thr Leu Tyr Glu Asn Leu
500 505 510

Phe Val Glu Lys Asn Met Lys Tyr Ile Pro Thr Glu Asp Val Ser Gly
515 520 525

Leu Ser Leu Glu Glu Gln Leu Arg Arg Leu Gln Glu Glu Arg Thr Cys
530 535 540

Lys Val Cys Met Asp Arg Glu Val Ser Ile Val Phe Ile Pro Cys Gly
545 550 555 560

His Leu Val Val Cys Gln Glu Cys Ala Pro Ser Leu Arg Lys Cys Pro
565 570 575

Ile Cys Arg Gly Thr Ile Lys Gly Thr Val Arg Thr Phe Leu Ser
580 585 590

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Met Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

AGTGCGGGTT TTTATTATGT G 21

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

AGATGACCAC AAGGAATAAA CACTA 25

What is claimed is:

1. A substantially pure nucleic acid encoding an IAP polypeptide.

2. The nucleic acid of claim 1, wherein said polypeptide comprises a ring zinc finger domain and at least one BIR domain.

3. The nucleic acid of claim 2, wherein said polypeptide has at least two BIR domains.

4. The nucleic acid of claim 3, wherein said polypeptide has at least three BIR domains.

5. The nucleic acid of claim 1, wherein said polypeptide comprises at least one BIR domain but lacks a ring zinc finger domain.

6. The nucleic acid of claim 5, wherein said polypeptide has at least two BIR domains.

7. The nucleic acid of claim 6, wherein said polypeptide has at least three BIR domains.

8. The nucleic acid of claim 1, wherein said polypeptide comprises a ring zinc finger domain but lacks a BIR domain.

9. The nucleic acid of claim 1, wherein said nucleic acid is mammalian.

10. The nucleic acid of claim 9, wherein said mammal is a human.

11. The nucleic acid of claim 9, wherein said DNA contains the m-xiap gene, the m-hiap-1 gene, or the m-hiap-2 gene.

12. The nucleic acid of claim 10, wherein said DNA contains the xiap gene, the hiap-1 gene, or the hiap-2 gene.

13. The nucleic acid of claim 1, wherein said nucleic acid is genomic DNA or cDNA.

14. A substantially pure DNA having the sequence of Fig. 1, or degenerate variants thereof, and encoding the amino acid sequence of Fig. 1, the sequence of Fig. 2, or degenerate variants thereof, and encoding the amino acid sequence of Fig. 2, the sequence of Fig. 3, or degenerate variants thereof, and encoding the amino acid sequence of Fig. 3, or the sequence of Fig. 4, or degenerate variants thereof, and encoding the amino acid sequence of Fig. 4.

15. Substantially pure DNA having about 50% or greater nucleotide sequence identity to the DNA sequence of Fig. 1, Fig. 2, Fig. 3, or Fig. 4.

16. A purified DNA sequence substantially identical to the DNA sequence shown in Fig. 1, Fig. 2, Fig. 3, or Fig. 4.

17. The DNA of claim 1, wherein said DNA is operably linked to regulatory sequences for expression of said polypeptide and wherein said regulatory sequences comprise a promoter.

18. The DNA of claim 17, wherein said promoter is a constitutive promoter, is inducible by one or more external agents, or is cell-type specific.

19. A vector comprising the DNA of claim 1, said vector being capable of directing expression of the peptide encoded by said DNA in a vector-containing cell.

20. A cell that contains the DNA of claim 1.

21. The cell of claim 20, said cell being present in a patient having a disease that is caused by excessive or insufficient cell death.

22. The cell of claim 20, said cell being selected from the group consisting of a fibroblast, a neuron, a glial cell, an insect cell, an embryonic stem cell, and a lymphocyte.

23. A transgenic cell that contains the DNA of claim 1, wherein said DNA is expressed in said transgenic cell.

24. A transgenic animal generated from the cell of claim 20, wherein said DNA is expressed in said transgenic animal.

25. A substantially pure mammalian IAP polypeptide, or fragment thereof.

26. The polypeptide of claim 25, said polypeptide being encoded by the nucleic acid of claim 5, claim 6, claim 7, or claim 8.

27. The polypeptide of claim 25, said polypeptide comprising an amino acid sequence substantially identical to an amino acid sequence shown in Fig. 1, Fig. 2, Fig. 3, or Fig. 4.

28. The polypeptide of claim 25, wherein said polypeptide is a mammalian polypeptide.

29. The polypeptide of claim 25, wherein said polypeptide is a human polypeptide.

30. The polypeptide of claim 28, wherein said polypeptide is M-XIAP, M-HIAP-1, or M-HIAP-2.

31. The polypeptide of claim 29, wherein said polypeptide is XIAP, HIAP-1, or HIAP-2.

32. A therapeutic composition comprising as an active ingredient an IAP polypeptide according to claim 25, said active ingredient being formulated in a physiologically acceptable carrier.

33. The composition of claim 32, said active ingredient being an IAP polypeptide encoded by the nucleic acid of claim 5, claim 6, claim 7, or claim 8.

34. A method of inhibiting apoptosis in a cell, said method comprising administering to said cell an apoptosis inhibiting amount of IAP polypeptide.

35. The method of claim 34, wherein said cell is in a mammal.

36. The method of claim 35, wherein said mammal is a human.

37. The method of claim 35, wherein said human has been diagnosed as being HIV-positive, or as having AIDS, a neurodegenerative disease, a myelodysplastic syndrome, or an ischemic injury.

38. The method of claim 37, wherein said ischemic injury is caused by a myocardial infarction, a stroke, a reperfusion injury, or a toxin-induced liver disease.

39. A method of inhibiting apoptosis in a mammal, said method comprising providing a transgene encoding an IAP polypeptide or fragment thereof to a cell of said mammal, said transgene being positioned for expression in said cell.

40. The method of claim 39 wherein said transgene encodes M-XIAP, M-HIAP-1, or M-HIAP-2.

41. The method of claim 39, wherein said mammal is a human.

42. The method of claim 41, wherein said polypeptide is XIAP, HIAP-1, or HIAP-2.

43. The method of claim 39, wherein said mammal is HIV-positive or has AIDS.

44. The method of claim 43, wherein said cell is a T cell.

45. The method of claim 44, wherein said T cell is a $CD4^+$ T cell.

46. The method of claim 39, wherein said mammal has a neurodegenerative disease.

47. The method of claim 39, wherein said mammal has an ischemic injury.

48. The method of claim 47, wherein said ischemic injury is caused by a myocardial infarction, a stroke, a reperfusion injury, or a toxin-induced liver disease.

49. A method of detecting an IAP gene in an animal cell, said method comprising contacting the DNA of claim 2, or a portion thereof that is greater than about 18 nucleotides in length, with a preparation of genomic DNA from said animal cell, said method providing detection of DNA sequences having about 50% or greater nucleotide sequence identity with the sequence of Fig. 1, Fig. 2,
Fig. 3, or Fig. 4.

50. A method of obtaining an IAP polypeptide, said method comprising:

(a) providing a cell with DNA encoding an IAP polypeptide, said DNA being positioned for expression in said cell;

(b) culturing said cell under conditions for expressing said DNA; and

(c) isolating said IAP polypeptide.

51. The method of claim 50, wherein said DNA further comprises a promotor inducible by one or more external agents.

52. The method of claim 45 wherein said IAP polypeptide is XIAP, HIAP-1, HIAP-2, M-XIAP, M-HIAP-1, or M-HIAP-2.

53. A method of isolating an IAP gene or portion thereof having sequence identity to xiap, m-xiap, hiap-1, m-hiap-1, hiap-2, or m-hiap-2, said method comprising amplifying by PCR said IAP gene or portion thereof using oligonucleotide primers wherein said primers

(a) are each greater than 13 nucleotides in length;

(b) each have regions of complementarily to opposite DNA strands in a region of the nucleotide sequence of either Fig. 1, Fig. 2, Fig. 3, or Fig. 4; and

(c) optionally contain sequences capable of producing restriction enzyme cut sites in the amplified product; and isolating said IAP gene or portion thereof.

54. A substantially pure polypeptide comprising a ring zinc finger domain having the sequence: Glu-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa2-Xaa1-Xaa1-Xaa1-Cys-Lys-Xaa3-Cys-Met-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa3-Xaa1-Phe-Xaa1-Pro-Cys-Gly- His-Xaa1-Xaa1-Xaa1-Cys-Xaa1-Xaa1-Cys-Ala-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Cys-Pro-Xaa1-Cys, wherein Xaa1 is any amino acid, Xaa2 is Glu or Asp and Xaa3 is Val or Ile.

55. The polypeptide of claim 54, further comprising at least one BIR domain having a copy of the sequence: Xaa1-Xaa1-Xaa1-Arg-Leu-Xaa1-Thr-Phe-Xaa1-Xaa1-Trp- Pro-Xaa2-Xaa1-Xaa1-Xaa2-Xaa2-Xaa1-Xaa1-Xaa1-Xaa1-Leu-Ala- Xaa1-Ala-Gly-Phe-Tyr-Tyr-Xaa1-Gly-Xaa1-Xaa1-Asp-Xaa1-Val-Xaa1-Cys-Phe-Xaa1-Cys-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Trp-Xaa1 Xaa1-Xaa1-Asp-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-His-

Xaa1-Xaa1-Xaa1-Xaa1-Pro-Xaa1-Cys-Xaa1-Phe-Val, wherein Xaa1 may be any amino acid and Xaa2 may be any amino acid or may be absent.

56. The polypeptide of claim 55, said polypeptide comprising at least two of said BIR domains.

57. The polypeptide of claim 56, said polypeptide comprising at least three of said BIR domains.

58. A recombinant IAP gene encoding the polypeptide of claim 54.

59. A method of isolating an IAP gene or fragment thereof from a cell, said method comprising:

(a) providing a sample of cellular DNA;

(b) providing a pair of oligonucleotides having sequence homology to a conserved region of an IAP gene;

(c) combining said pair of oligonucleotides with said cellular DNA sample under conditions suitable for polymerase chain reaction-mediated DNA amplification; and

(d) isolating said amplified IAP gene or fragment thereof.

60. The method of claim 59, wherein said amplification is carried out using a reverse-transcription polymerase chain reaction.

61. The method of claim 60, wherein said reverse-transcription polymerase chain reaction is RACE.

62. A method of identifying an IAP gene in a mammalian cell, said method comprising:

(a) providing a preparation of mammalian cellular DNA;

(b) providing a detectably-labelled DNA sequence having homology to a conserved region of an IAP gene;

(c) contacting said preparation of cellular DNA with said detectably-labelled DNA sequence under hybridization conditions that provide detection of genes having 50% or greater nucleotide sequence identity; and

(d) identifying an IAP gene by its association with said detectable label.

63. The method of claim 62, wherein said DNA sequence is produced according to the method of claim 53.

64. A method of isolating an IAP gene from a recombinant DNA library, said method comprising:

(a) providing a recombinant DNA library;

(b) contacting said recombinant DNA library with a detectably-labelled gene fragment produced according to the method of claim 49 under hybridization conditions that provide for detection of genes having 50% or greater nucleotide sequence identity; and

(c) isolating a member of an IAP gene by its association with said detectable label.

65. A method of isolating an IAP gene from a recombinant DNA library, said method comprising:

(a) providing a recombinant DNA library;

(b) contacting said recombinant DNA library with a detectably-labelled oligonucleotide of any of claim 49 under hybridization conditions that provide for detection of genes having 50% or greater nucleotide sequence identity; and

(c) isolating an IAP gene by its association with said detectable label.

66. A recombinant mammalian polypeptide capable of inhibiting apoptosis wherein said polypeptide comprises a ring zinc finger sequence: Glu-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa2-Xaa1-Xaa1-Xaa1-Cys-Lys-Xaa3-Cys-Met-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa3-Xaa1-Phe-Xaa1-Pro-Cys-Gly-His-Xaa1-Xaa1- Xaa1-Cys-Xaa1-Xaa1-Cys-Ala-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Cys-Pro Xaa1-Cys, wherein Xaa1 and amino acid, Xaa2 is Glu or Asp and Xaa3 is Val or Ile;

and at least one BIR domain having the sequence Xaa1-Xaa1-Xaa1-Arg-Leu-Xaa1-Thr-Phe-Xaa1-Xaa1-Trp-Pro-Xaa2- Xaa1-Xaa1-Xaa2-Xaa2-Xaa1-Xaa1-Xaa1-Xaa1-Leu-Ala-Xaa1-Ala-Gly-Phe-Tyr-Tyr-Xaa1-Gly-Xaa1-Xaa1-Asp-Xaa1-Val-Xaa1-Cys-Phe-Xaa1-Cys-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Trp-Xaa1-Xaa1- Xaa1-Asp-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-His-Xaa1-Xaa1-Xaa1-Xaa1- Pro-Xaa1-Cys-Xaa1-Phe-Val, wherein Xaa1 may be any amino acid and Xaa2 is any amino acid or is absent.

67. An IAP gene isolated according to a method comprising:

(a) providing a sample of cellular DNA;

(b) providing a pair of oligonucleotides having sequence homology to a conserved region of an IAP disease-resistance gene;

(c) combining said pair of oligonucleotides with said cellular DNA sample under conditions suitable for polymerase chain reaction-mediated DNA amplification; and

(d) isolating said amplified IAP gene or fragment thereof.

68. An IAP gene isolated according to the method comprising:

(a) providing a preparation of cellular DNA;

(b) providing a detectably-labelled DNA sequence having homology to a conserved region of an IAP gene;

(c) contacting said preparation of cellular DNA with said detectably-labelled DNA sequence under hybridization conditions providing detection of genes having 50% or greater nucleotide sequence identity; and

(d) identifying an IAP gene by its association with said detectable label.

69. A method of identifying an IAP gene, said method comprising:

(a) providing a mammalian cell sample;

(b) introducing by transformation into said cell sample a candidate IAP gene;

(c) expressing said candidate IAP gene within said cell sample; and

(d) determining whether said sample exhibits an altered level of apoptosis whereby an alteration in the level of apoptosis identifies an IAP gene.

70. The method of claim 69, wherein said cell sample is selected from the group consisting of a lymphocyte, a fibroblast, an insect cell, a glial cell, an embryonic stem cell, and a neuron.

71. The method of claim 69, wherein said candidate IAP gene is obtained from a cDNA expression library.

72. An IAP gene isolated according to the method comprising:

(a) providing a cell sample;

(b) introducing by transformation into said cell sample a candidate IAP gene;

(c) expressing said candidate IAP gene within said cell sample; and

(d) determining whether said cell sample exhibits a decreased apoptosis response, whereby a decreased level of apoptosis identifies an IAP gene.

73. A purified antibody that binds specifically to an IAP family polypeptide.

74. A method of identifying a compound that modulates apoptosis, said method comprising:

(a) providing a cell expressing an IAP polypeptide; and

(b) contracting said cell with a candidate compound and monitoring the expression of an IAP gene, an alteration in the level of expression of said gene indicating the presence of a compound which modulates apoptosis.

75. The method of claim 74, wherein said IAP gene is xiap, hiap-1, hiap-2, m-xiap, m-hiap-1, or m-hiap-2.

76. The method of claim 74, wherein said cell is a lymphocyte, said IAP is selected from the group consisting of hiap-1 and hiap-2, and said modulating is an increase in hiap-1 or hiap-2 expression.

77. A method of diagnosing a mammal for the presence of an apoptosis disease or an increased likelihood of developing a disease involving apoptosis in a mammal, said method comprising isolating a sample of nucleic acid from said mammal and determining whether said nucleic acid comprises an IAP mutation, said mutation being an indication that said mammal has an apoptosis disease or an increased likelihood of developing a disease involving apoptosis.

78. A method of diagnosing a mammal for the presence of an apoptosis disease or an increased likelihood of developing an apoptosis disease, said method comprising measuring IAP gene expression in a sample from said mammal, an alteration in said expression relative to a sample from an unaffected mammal being an indication that said mammal has an apoptosis disease or increased likelihood of developing an apoptosis disease.

79. The method of claim 77 or 78, wherein said IAP gene is xiap, hiap-1, hiap-2, m-xiap, m-hiap-1, or m-hiap-2.

80. The method of claim 77 or 78, wherein said gene expression is measured by assaying the amount of IAP polypeptide in said sample.

81. The method of claim 80, wherein said IAP polypeptide is measured by immunological methods or by assaying the amount of IAP RNA in said sample.

82. A kit for diagnosing a mammal for the presence of an apoptosis disease of an increased liklihood of developing an apoptosis disease, said kit comprising a substantially pure antibody that specifically binds an IAP polypeptide.

83. The kit of claim 82, further comprising a means for detecting said binding of said antibody to said IAP polypeptide.

84. The method of claim 34, said method comprising administering to said cell an apoptosis inhibiting amount of the polypeptide of claim 8.

85. A method of inducing apoptosis in a cell, said method comprising administering to said cell a negative regulator of the IAP-dependent anti-apoptotic pathway.

86. The method of claim 85, wherein said negative regulator is an IAP polypeptide comprising a ring zinc finger, but lacking at least one BIR domain.

87. The method of claim 85, wherein said cell is transfected with a gene encoding the IAP polypeptide of claim 8.

88. The method of claim 85, wherein said negative regulator is a purified antibody or a fragment thereof that binds specifically to an IAP polypeptide.

89. The method of claim 88, wherein said antibody specifically binds an approximately 26 kDa cleavage product of an IAP polypeptide, said cleavage product comprising at least one BIR domain but lacking a ring zinc finger domain

90. The method of claim 85, wherein said negative regulator is an IAP antisense mRNA molecule.

91. An IAP nucleic acid for use in modulating apoptosis.

92. An IAP polypeptide for use in modulating apoptosis.

93. The use of an IAP polypeptide for the manufacture of a medicament for the modulation of apoptosis.

94. The use of an IAP nucleic acid for the manufacture of a medicament for the modulation of apoptosis.

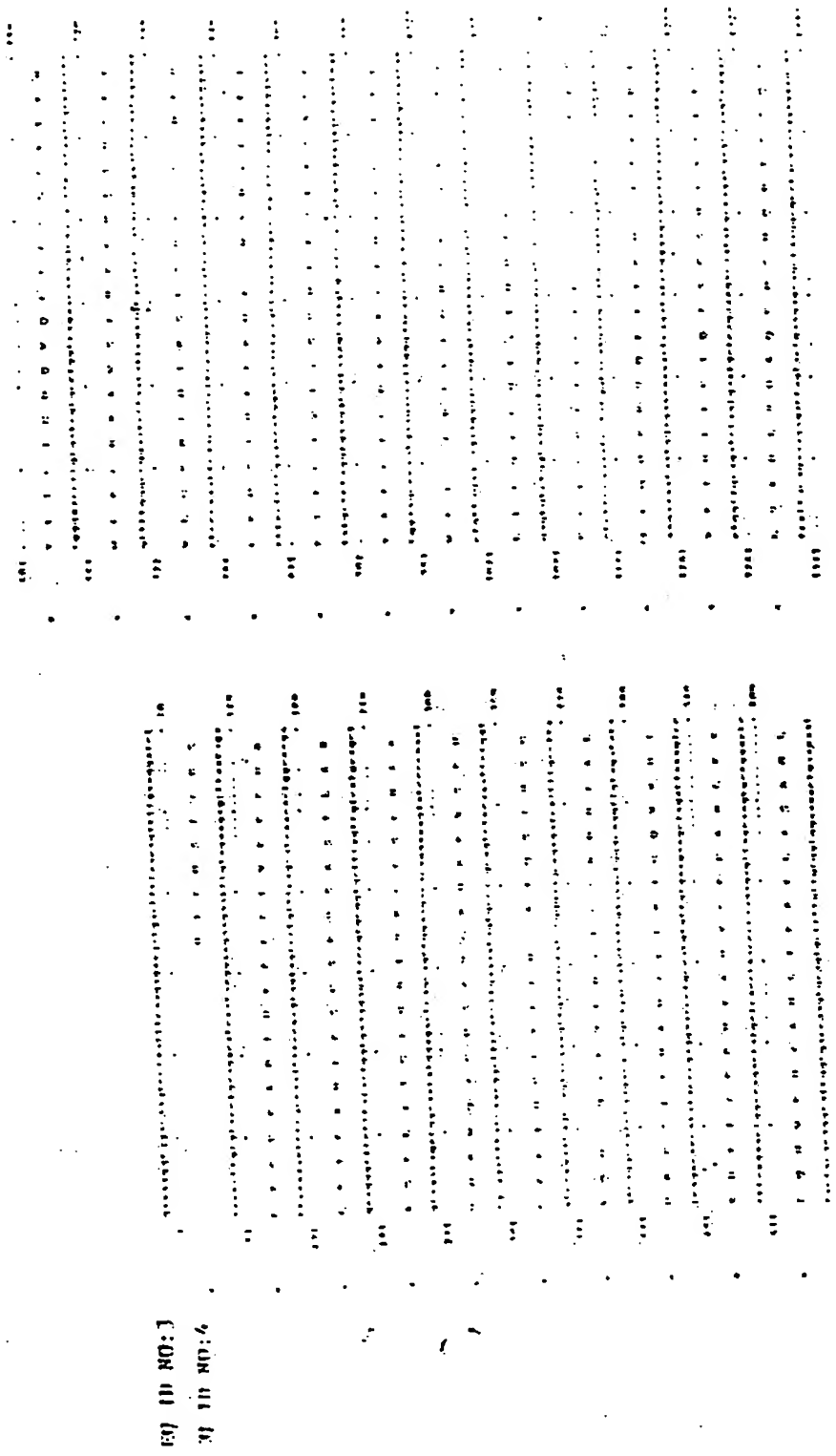


FIG. 1 (pp. 1 of 2)

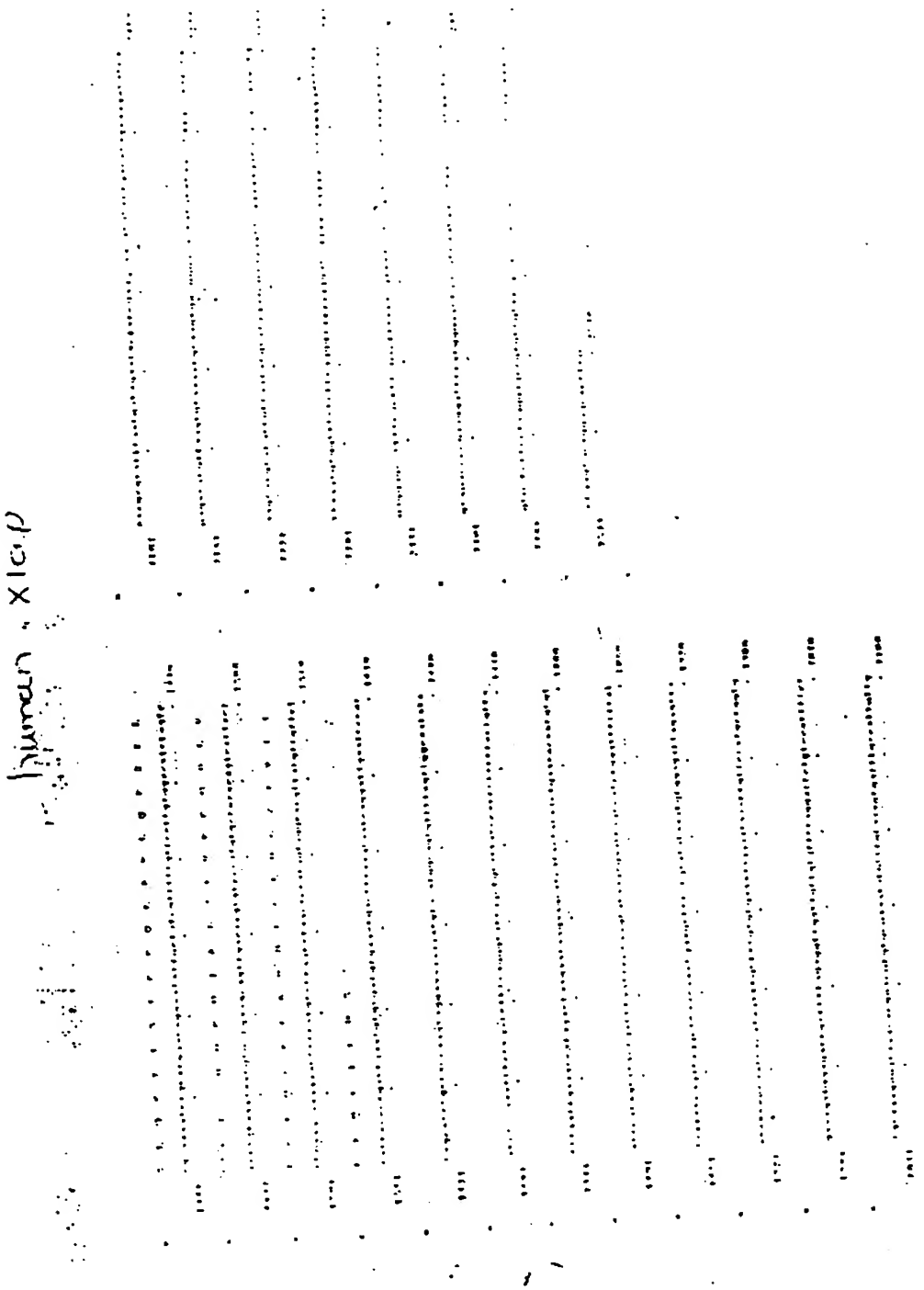


FIG. 1 (sheet 2 of 71)

human hlap7

SEQ ID NO:5

SEQ ID NO:6

FIG. 2 (pg. 1 of 7)

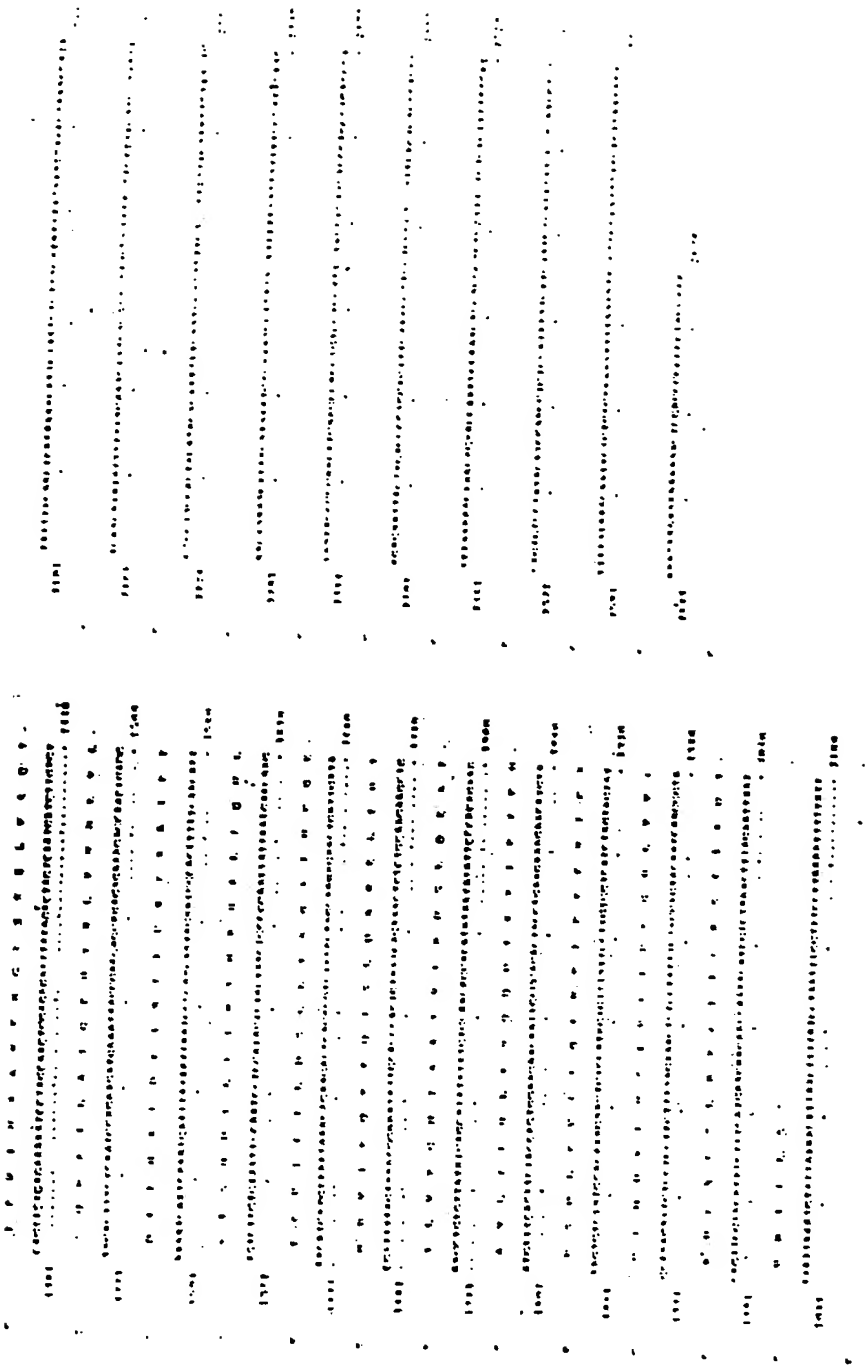


FIG. 2 (SHEET 2 of 3)

human trap'

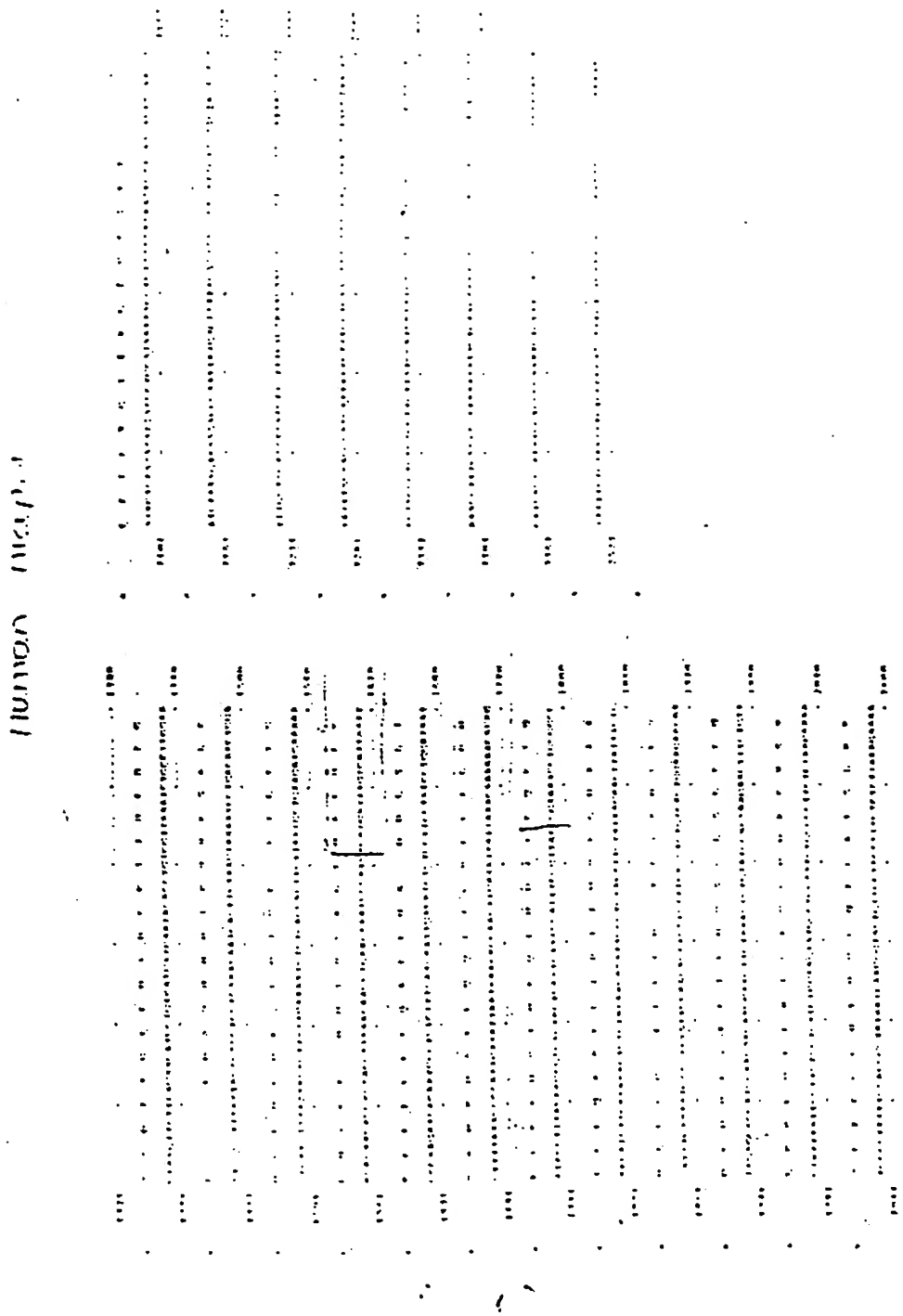
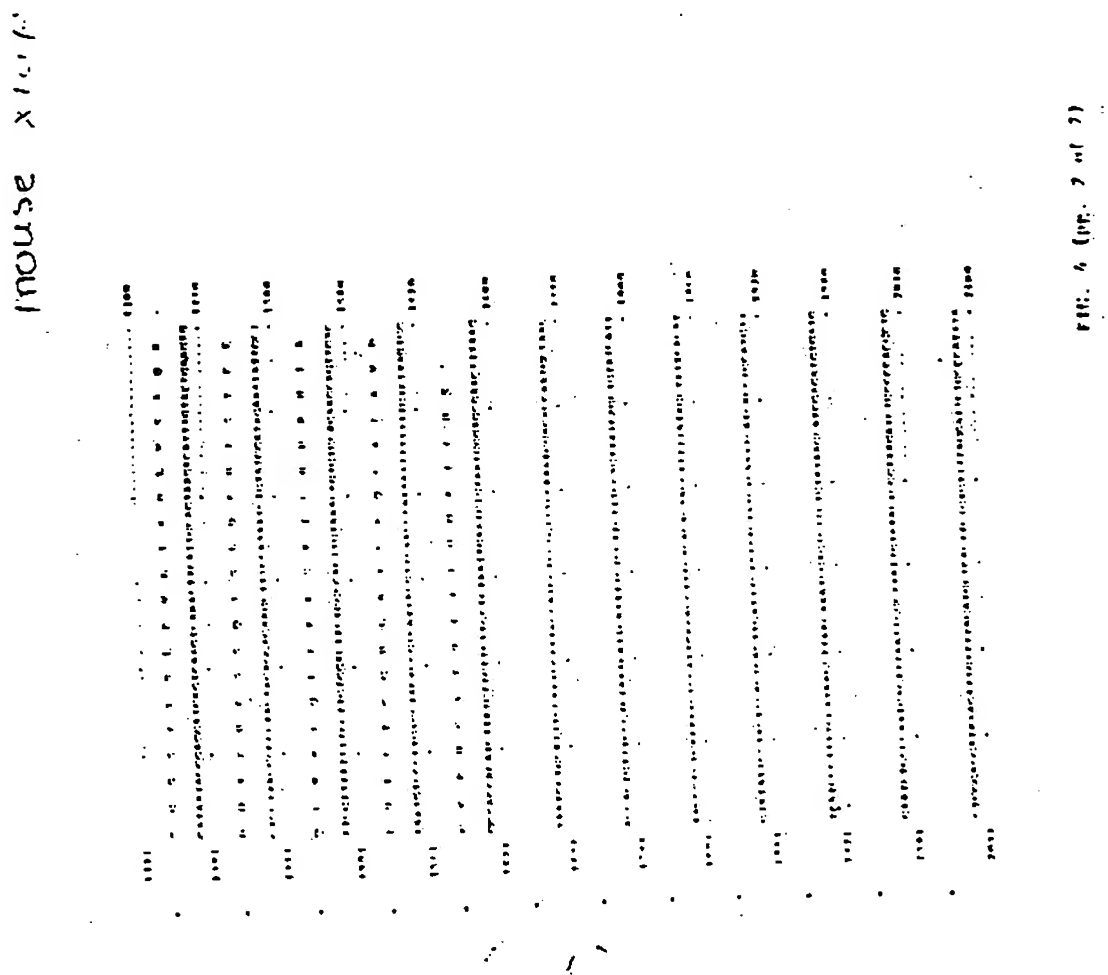


FIG. 3 (pg. 2 of 2)

mouse XIAP

FIG. 6 (1 of 2)



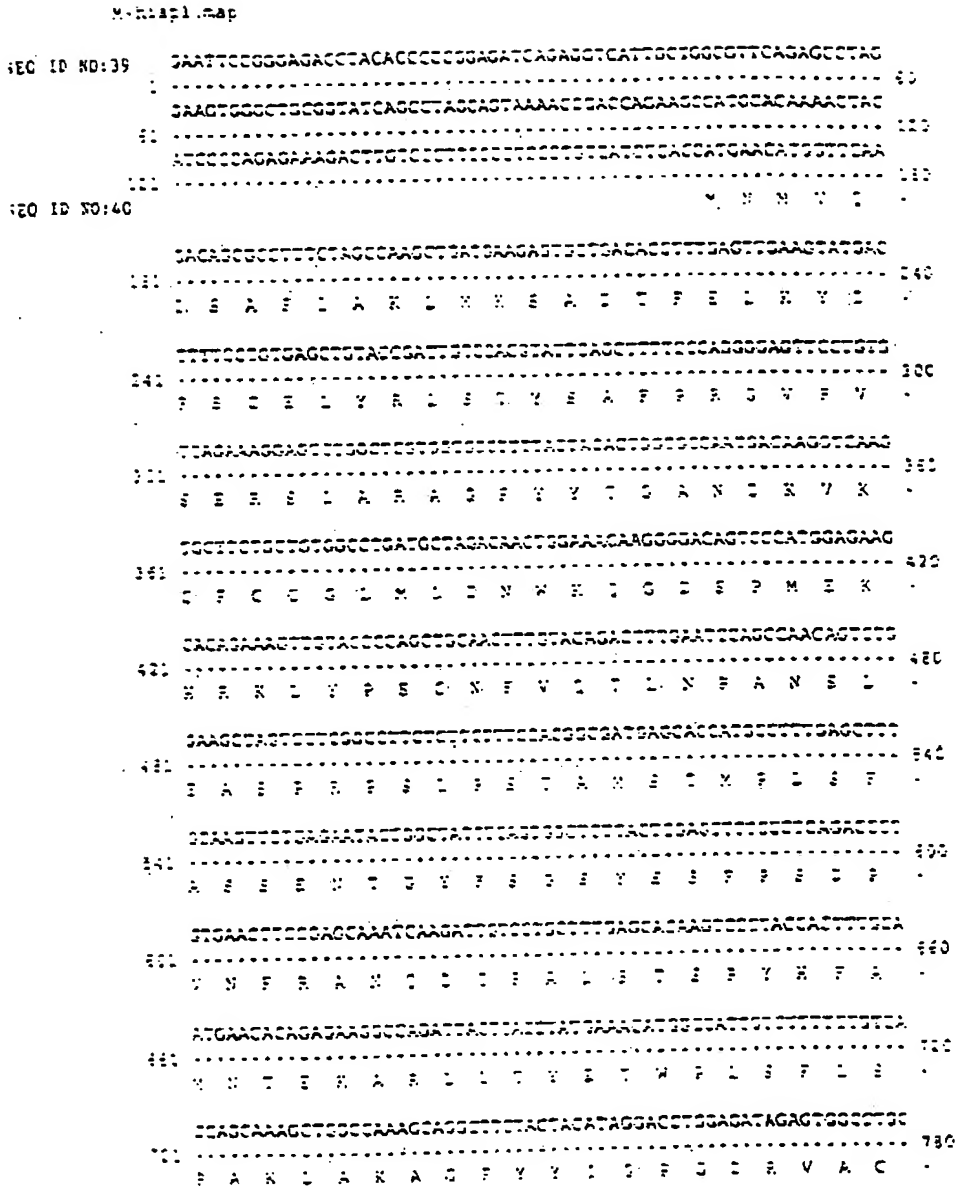


Fig. 5 (page 1 of 3)

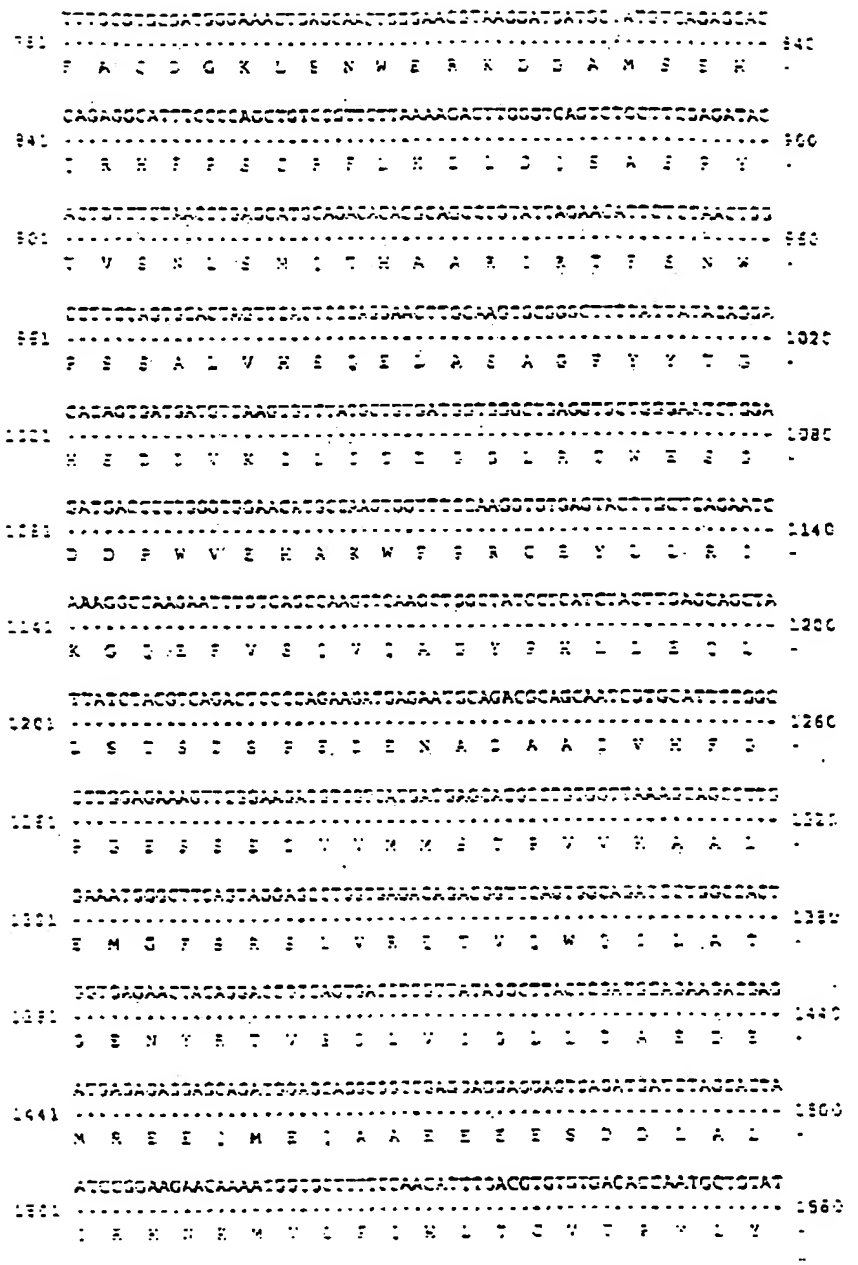


Fig. 5 (page 2 of 3)

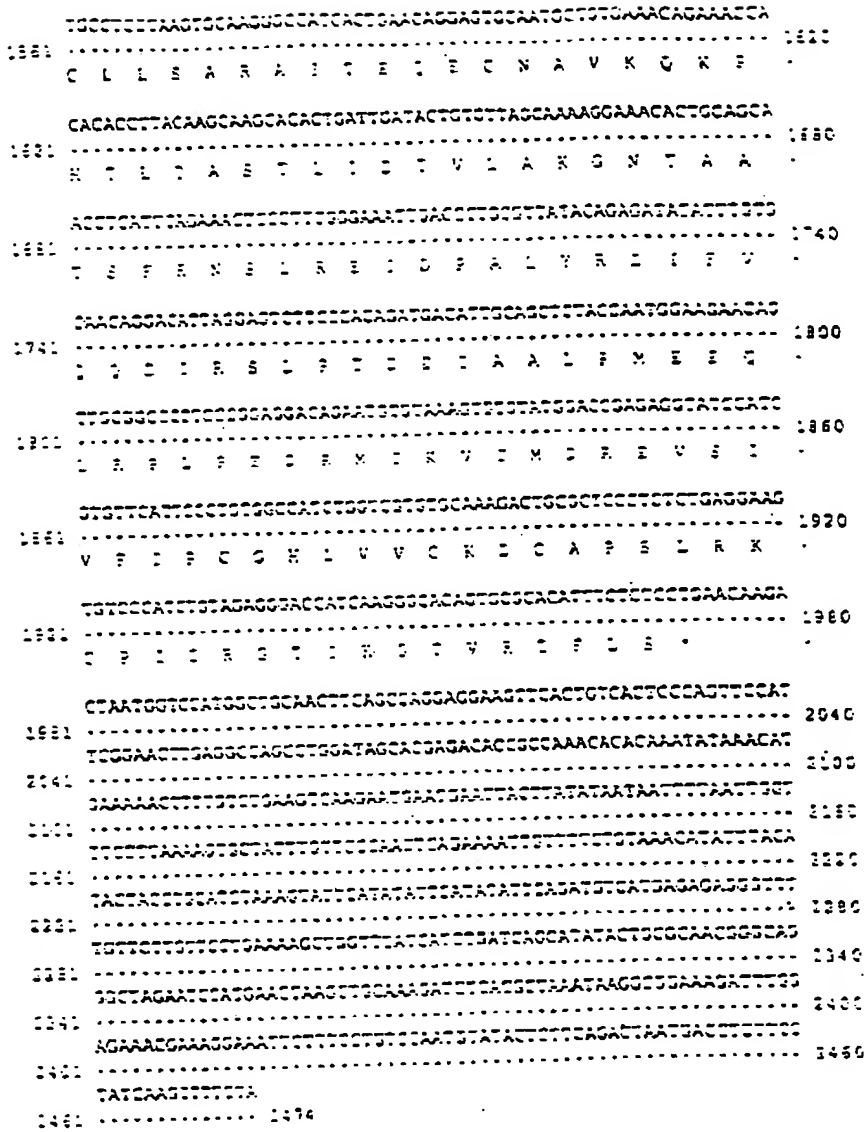


Fig. 5 (page 3 of 3)

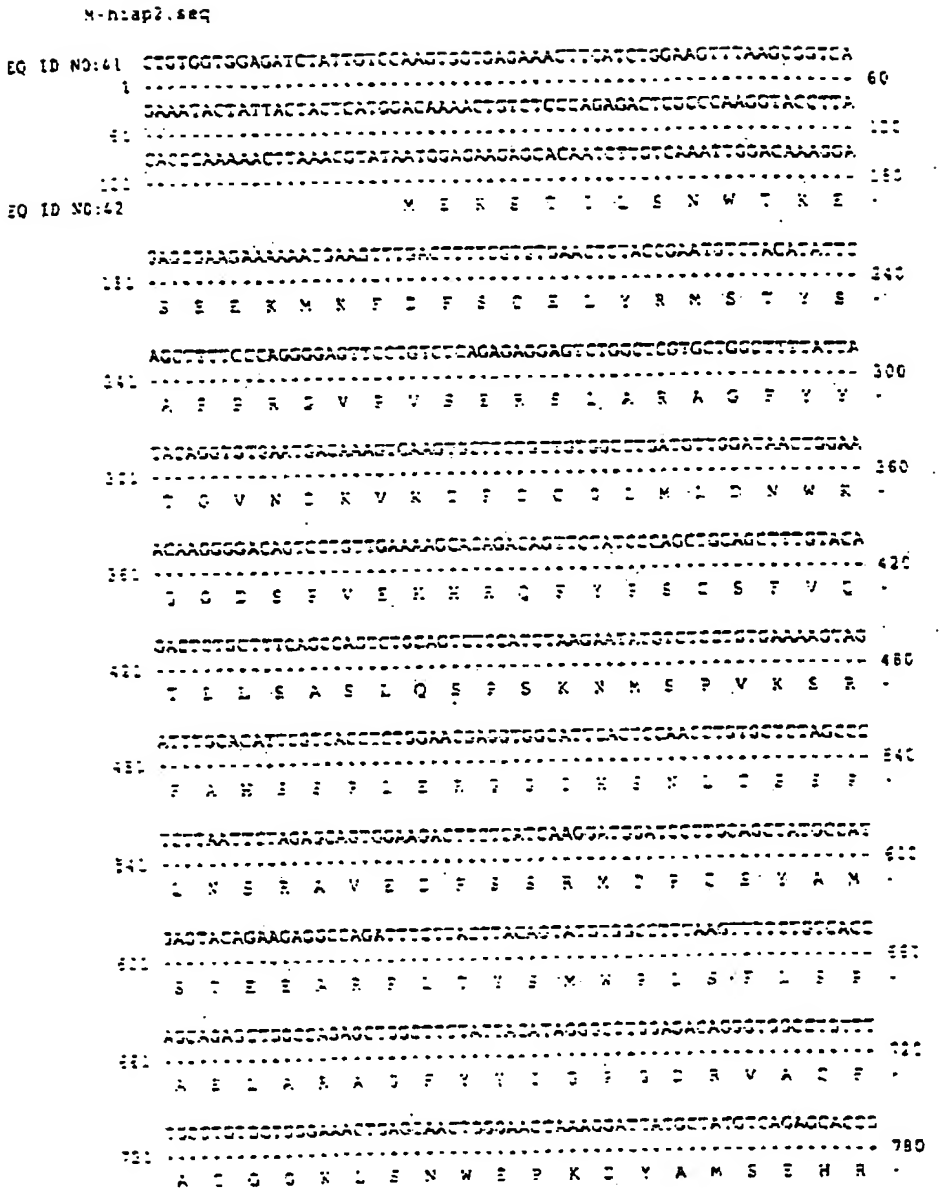


Fig. 6 (page 1 of 3)

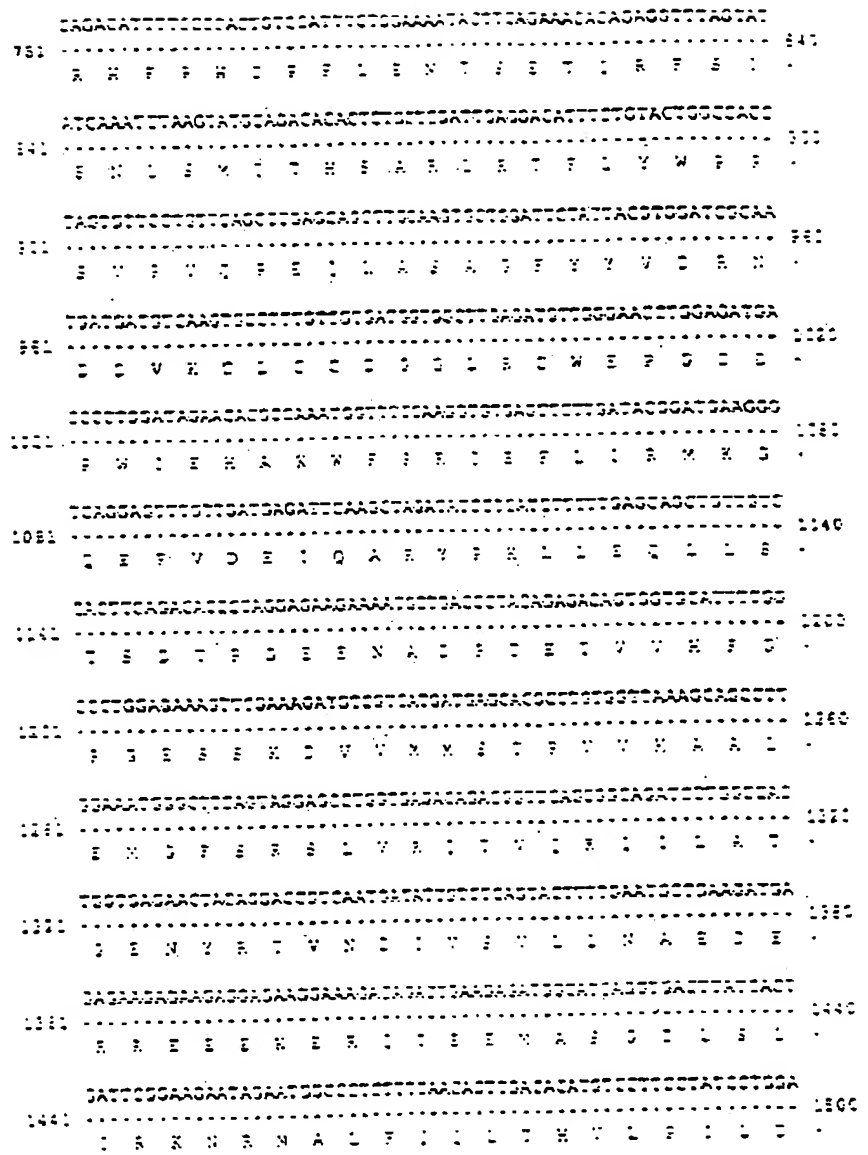


Fig. 6 (page 2 of 3)

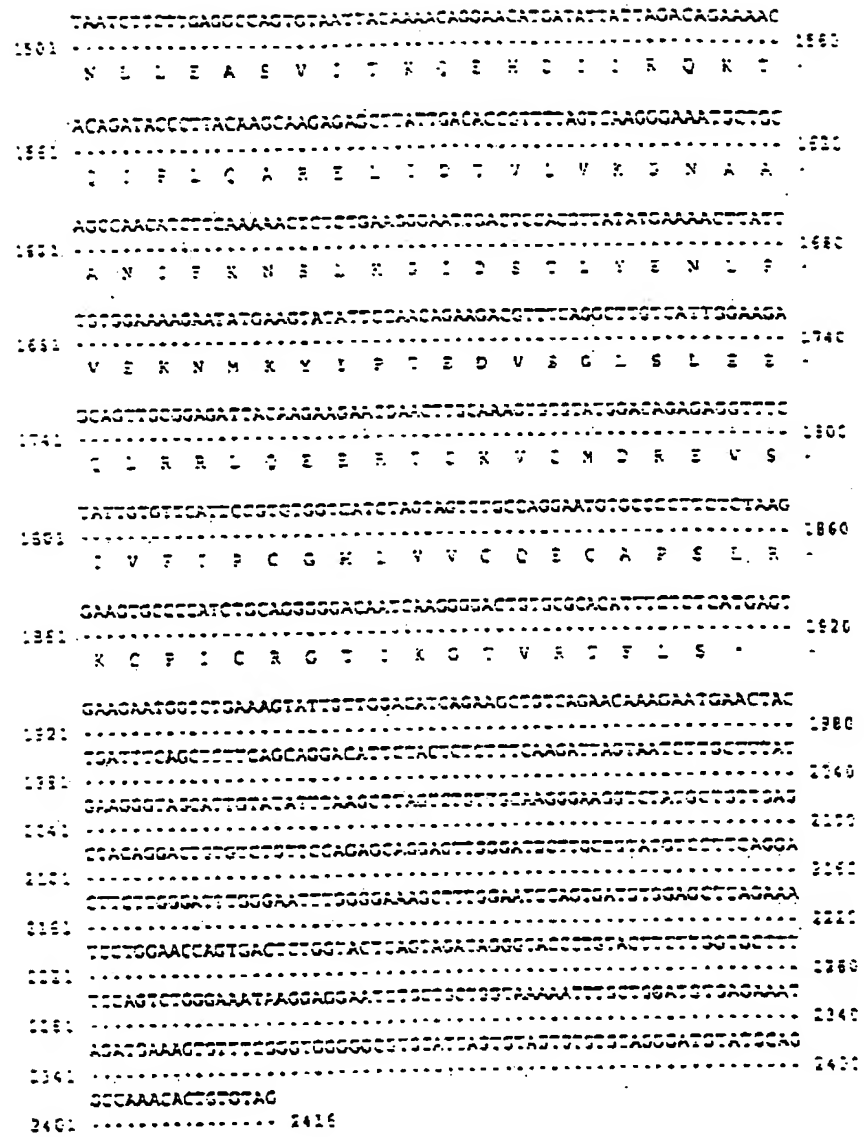


Fig. 6 (page 3 of 3)

Alignment of BIR (Baculovirus IAP Repeat) Domains

baculovirus
- Cp_iap — Cydia pomonella
- Op_iap — Orgyia pseudotsugata

human
- xiap — XIAP on X chromosome
- hiap1, hiap2 — two different human IAP genes

louse
- m-hiap — mouse homologue of human xiap gene

insect
- diap — Drosophila IAP gene, not clearly a homologue of xiap or hiap

Gaps on consensus. The consensus line represents amino acids or very similar amino acids which are present in 14 of the 15 BIR sequences at each position. Capitalized residues are those that are in the consensus sequence.

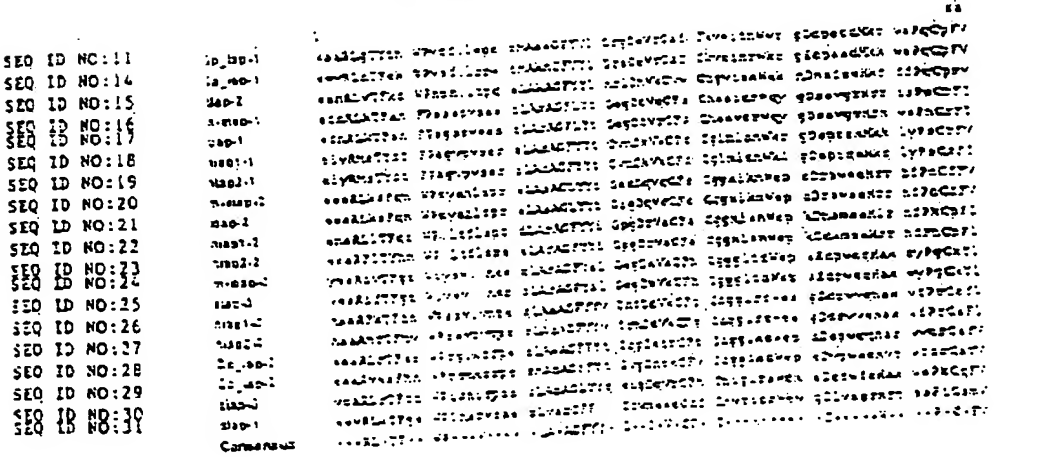


Fig. 7

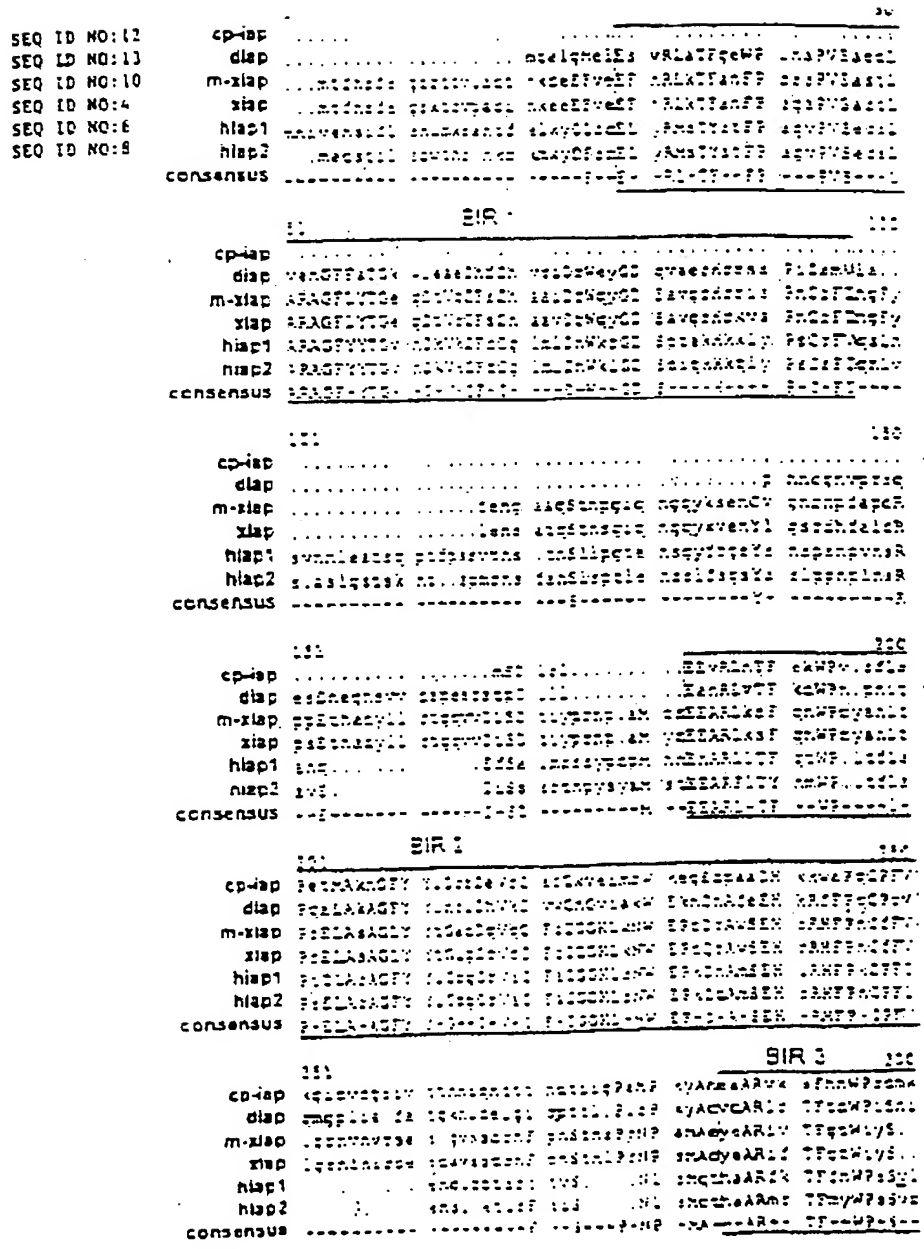


Fig. A (page 1 of 3)

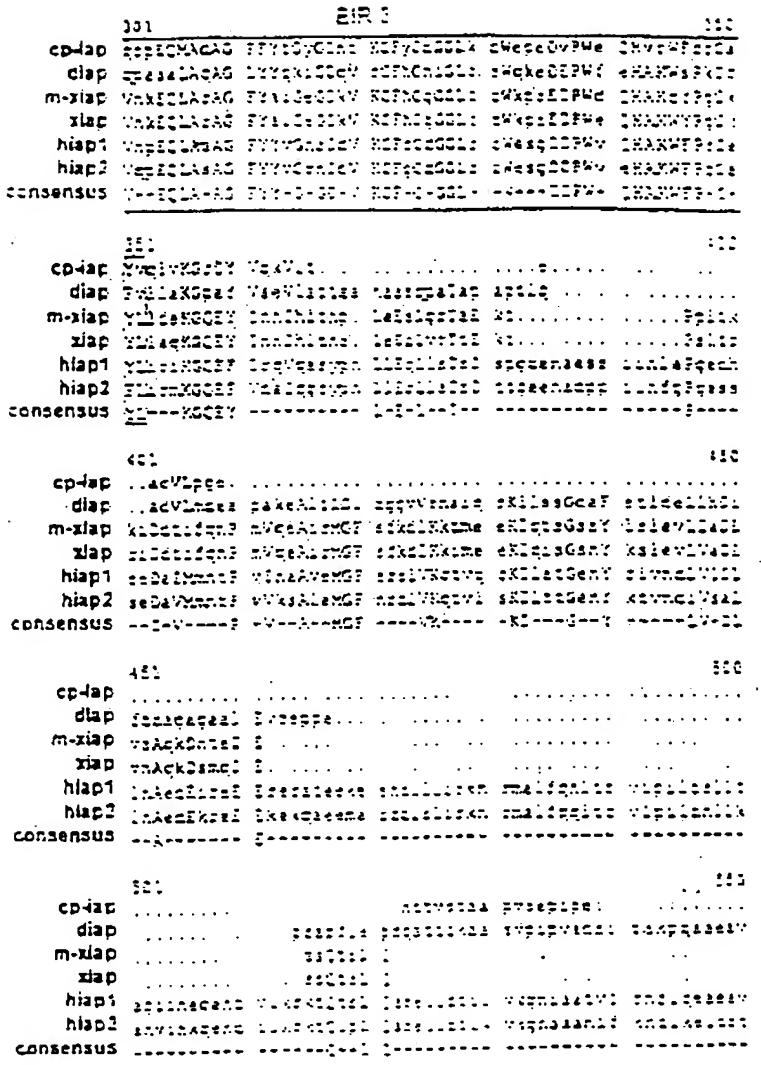


Fig. 8 (page 2 of 3)

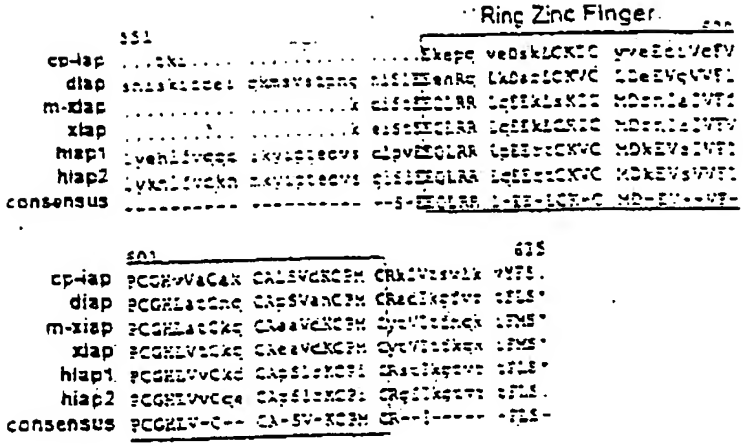


Fig. 8 (page 1 of 3)

Alignment of RBR (RING Zinc Finger) Domains

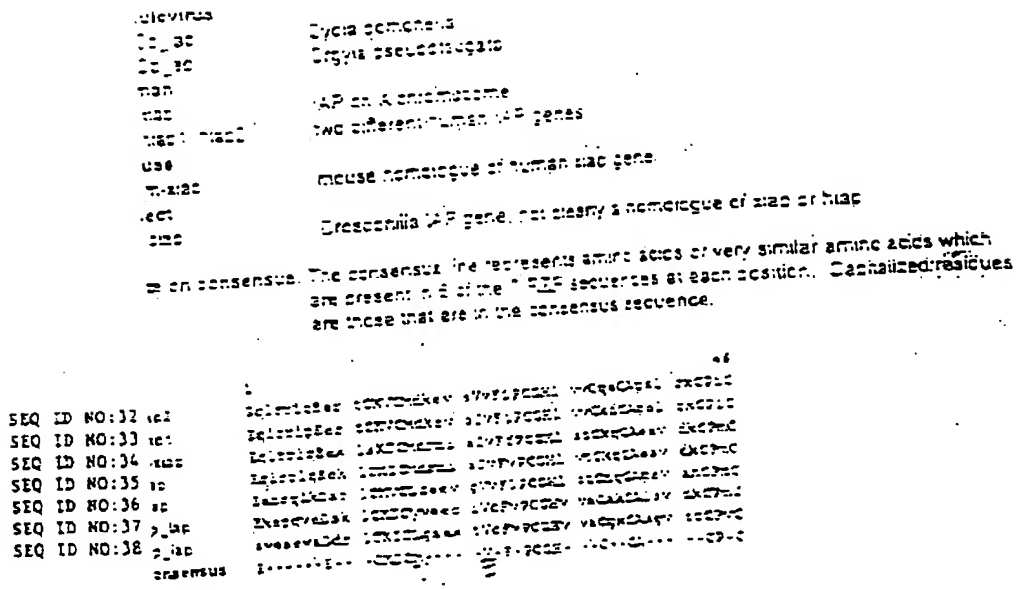
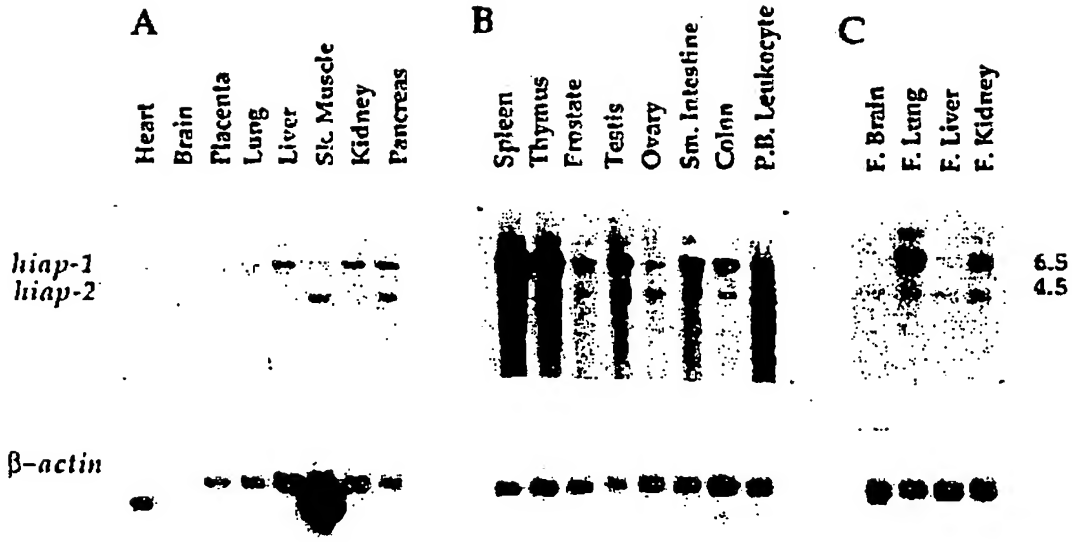


Fig. 9

FIG. 10



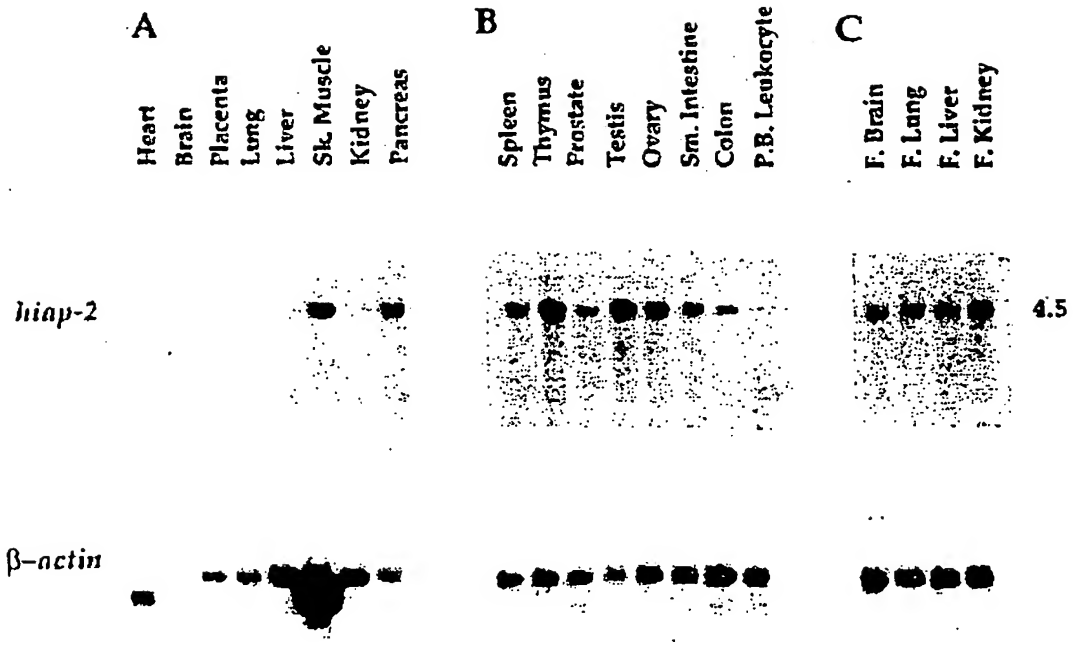

A
Heart
Brain
Placenta
Lung
Liver
Sk. Muscle
Kidney
Pancreas
B
Spleen
Thymus
Prostate
Testis
Ovary
Sm. Intestine
Colon
P.B. Leukocyte
C
F. Brain
F. Lung
F. Liver
F. Kidney
hiap-2
4.5
β-actin

FIG. 11

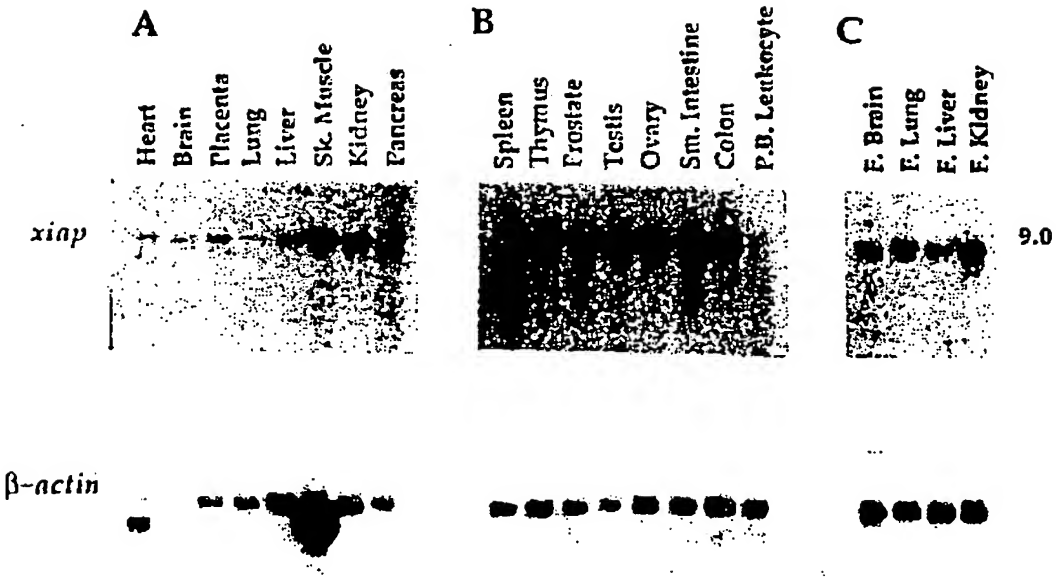


FIG. 12

13A

13B

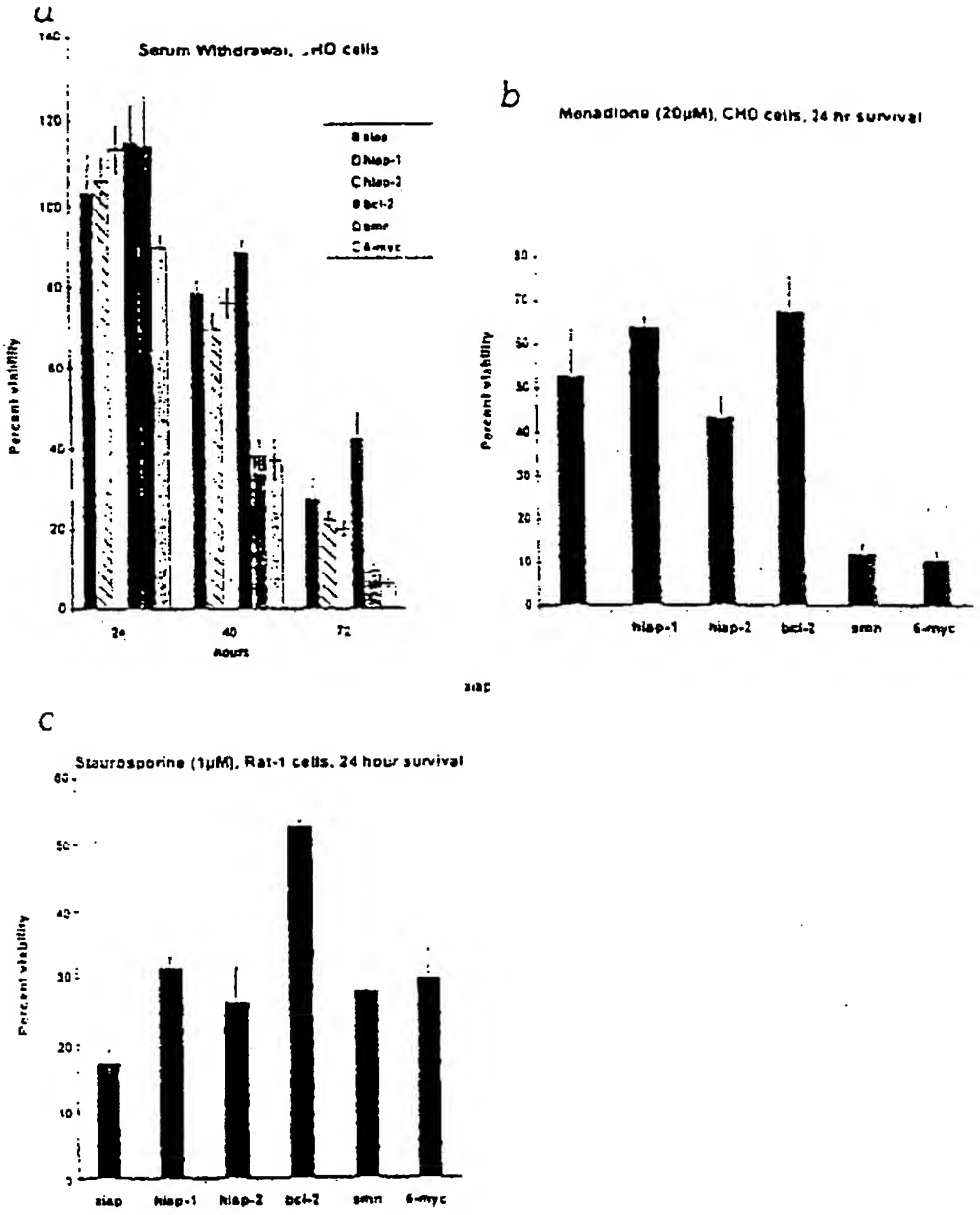

a
Serum Withdrawal, CHO cells
Percent viability
Hours
b
Menadione (20μM), CHO cells, 24 hr survival
Percent viability
xiap
hiap-1
hiap-2
bcl-2
smn
c-myc
c
Staurosporine (1μM), Rat-1 cells, 24 hour survival
Percent viability
xiap
hiap-1
hiap-2
bcl-2
smn
c-myc

Fig. 16A - C

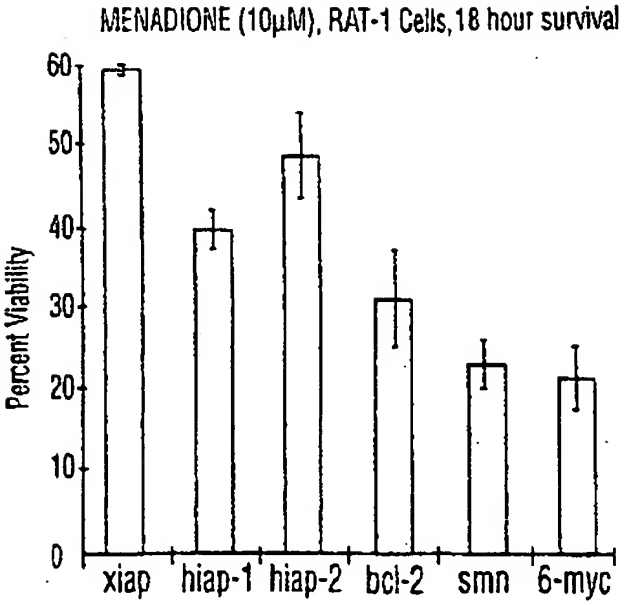


FIG.14D

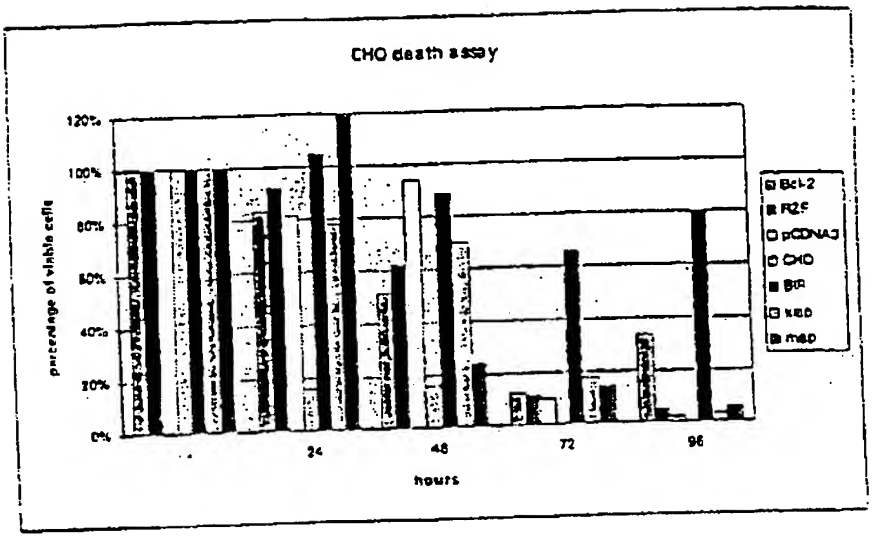

CHO death assay
120%
100%
80%
60%
40%
20%
0%
percentage of viable cells
24
48
72
96
hours
Bcl-2
R2F
pCDNA3
CHO
Bir
xiap
map

Figure 15A

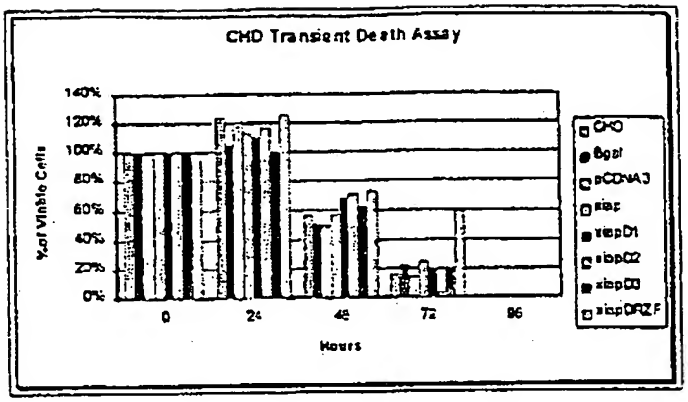

CHO Transient Death Assay
% of Viable Cells
140%
120%
100%
80%
60%
40%
20%
0%
0
24
48
72
96
Hours
CHO
6gpt
pCDNA3
xbp
xbpD1
xbpD2
xbpD3
xbpDRZF

Figure 15B

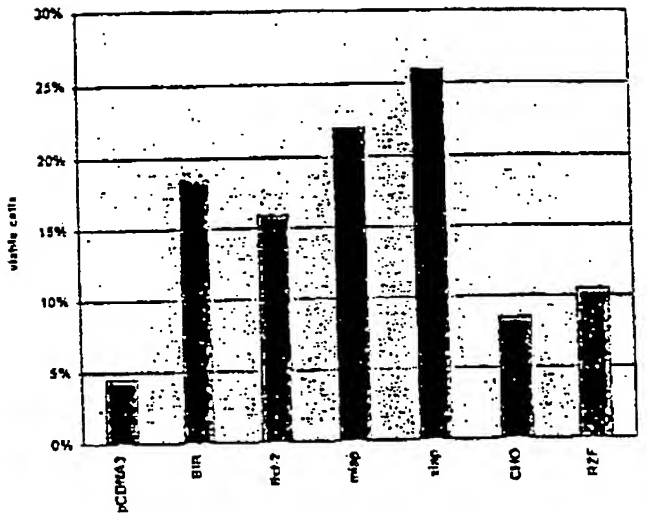

30%
25%
20%
15%
10%
5%
0%
viable cells
pCDNA3
BIR
Bcl-2
mlap
vlap
CIAO
R2F

Figure 16A

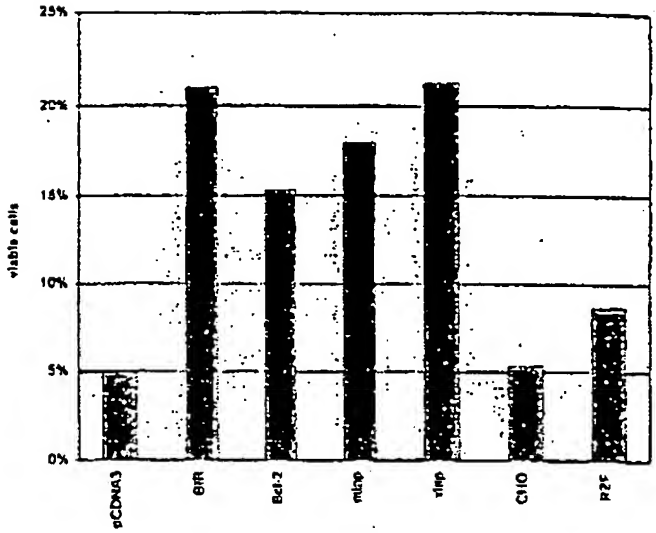


Figure 16B

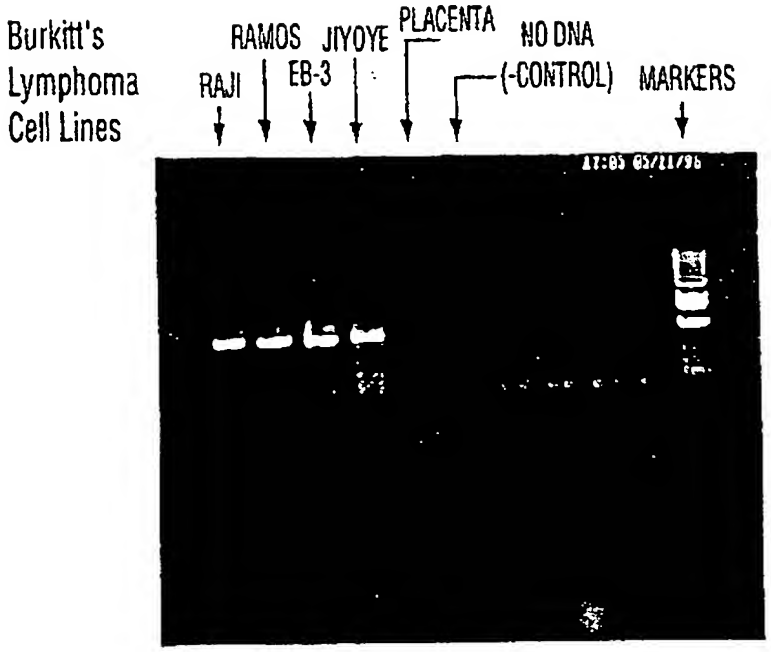


FIG. 17

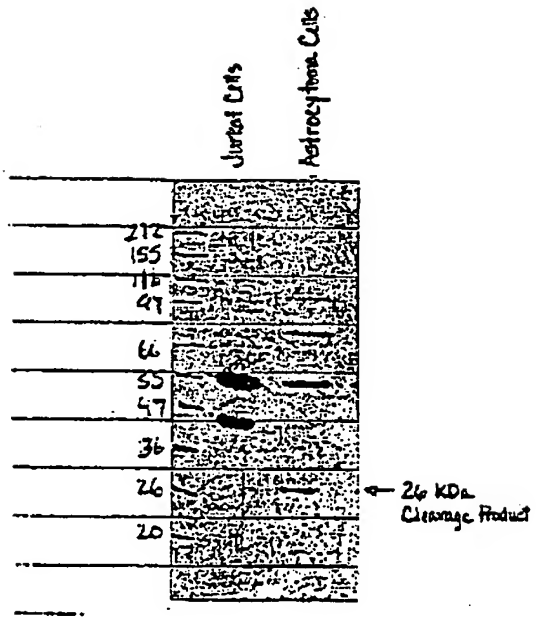

Jurkat Cells
Astrocytoma Cells
212
155
116
97
66
55
47
36
26
20
← 26 kDa
Cleavage Product

Figure 18

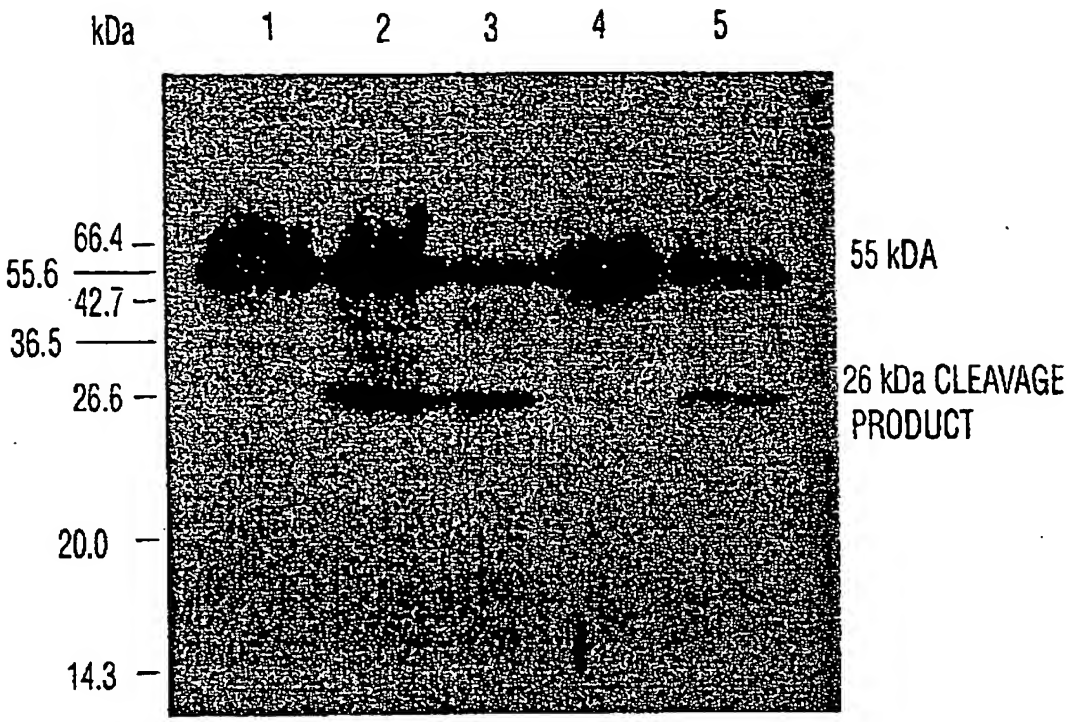


FIG.19

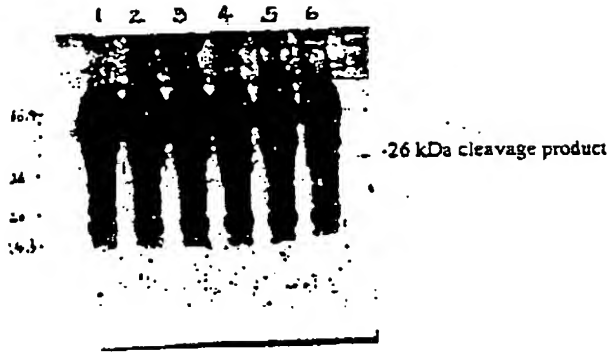


Figure 20

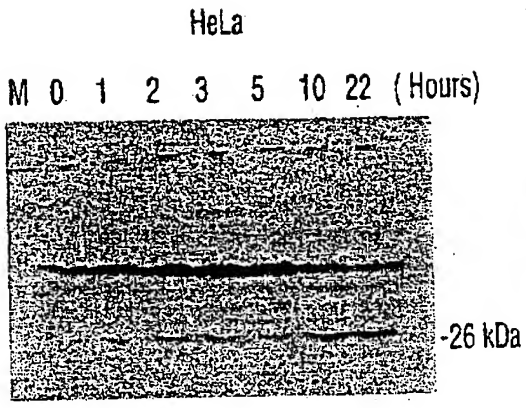


FIG. 21A

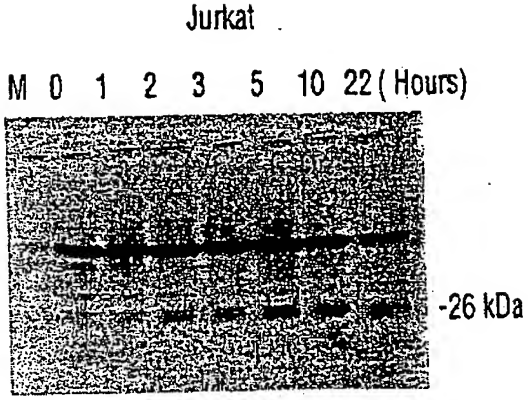


FIG. 21B

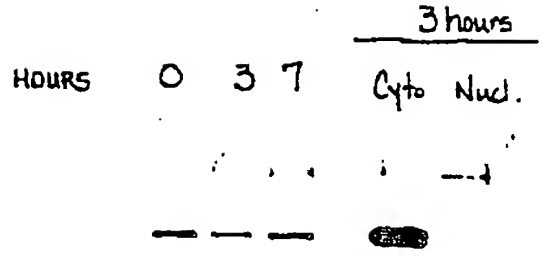


Figure 22A

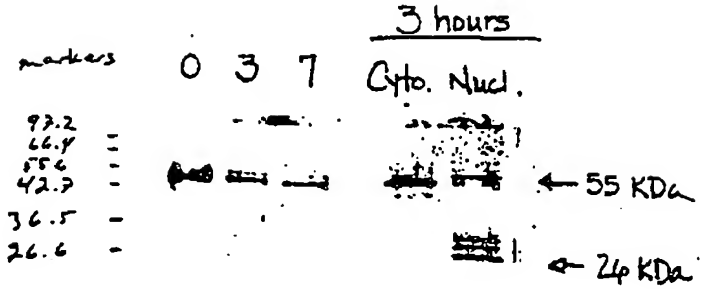


Figure 22B

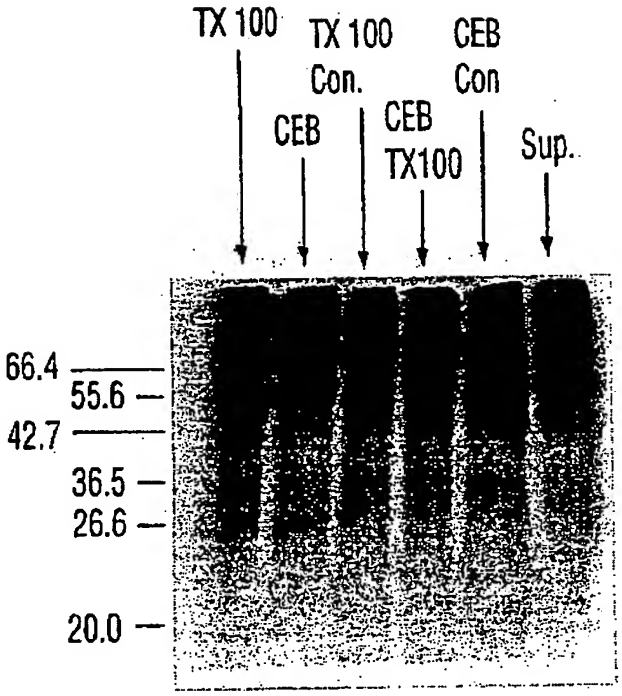

TX 100
TX 100 Con.
CEB Con
CEB
CEB TX100
Sup.
66.4
55.6
42.7
36.5
26.6
20.0

FIG. 23

SMART & BIGGAR

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76962-1

9 January 1997

International Searching Authority
European Patent Office
P.B. 5818 Patenlaan 2
NL-2280 HV Rijswijk
NETHERLANDS

Dear Sir:

Re: PCT Patent Application
Serial No. PCT/IB96/01022
University of Ottawa
(Robert G. Korneluk, et al)

We refer to the Invitation to Correct Defects dated October 3, 1996, copy attached, and in particular to the additional observations referred to therein. The Receiving Office of the International Bureau has objected to the poor quality of printing of the drawings, and has directed applicant to Rule 91 relating to the rectification of obvious errors. It is our belief that the authorized International Searching Authority to whom our request for rectification of obvious errors should be directed is ISA/EP.

This letter is filed pursuant to Rule 91.1(e)(ii) to request authorization by the International Searching Authority of rectification of obvious errors in the drawings. Such a request should have been filed within 17 months of the priority date.

However, we have only recently been appointed agent with respect to this application, and even more recently become aware of the Invitation to Correct Defects and the requirements therein relating to drawings. Since becoming aware of the situation, we have been diligently pursuing the matter with applicant's US patent attorney and applicant's licensee. The drawings originally filed were apparently photocopies of drawings prepared for the priority application, thus the relatively poor quality. It was necessary to locate the original versions of each of the drawings, some of which were with the US patent attorney and some of which were with the inventors. Fresh formal drawings meeting the PCT requirements then had to be prepared from these originals. All of these events were further delayed by the holiday season.

We have now completed preparation of amended drawings. These have been filed in the International Bureau Receiving Office, in response to the outstanding Invitation to Correct Defects, a response to which is due January 10, 1997. We enclose copies of our letter to the International Bureau together with the amended sheets proposed for replacement.

We understand that a request for rectification of an obvious error may be entertained under Rule 91.1 so long as technical preparations for international publication have not yet been completed. We accordingly request that the International Searching Authority consider this matter before completing technical preparations for publication, and grant the requested request for rectification.

The "errors" in the drawings appear to reside in the fact that their quality is such that in at least some respects they are not clearly readable. Although the quality of the drawings originally filed admitedly is poor, a comparison of the drawings originally filed with the enclosed amended drawings reveals that the content is obviously the same. Further with respect to those of the Figures which refer to sequence listings, the content of the amended drawings can be verified by a comparison with the print copy of the sequence listing which accompanied the application when filed and which forms part of the description. For these reasons, applicant respectfully submits that the "errors" in the drawings are obvious in that anyone would immediately realize that nothing else could have been intended other than what is offered as rectification.

In all of these circumstances, we request that the International Searching Authority authorize rectification of obvious errors in the drawings, and acceptance of the replacement sheets submitted herewith.

Yours very truly,

SMART & BIGGAR

(Mrs.) Joy D. Morrow

HUMAN xiap

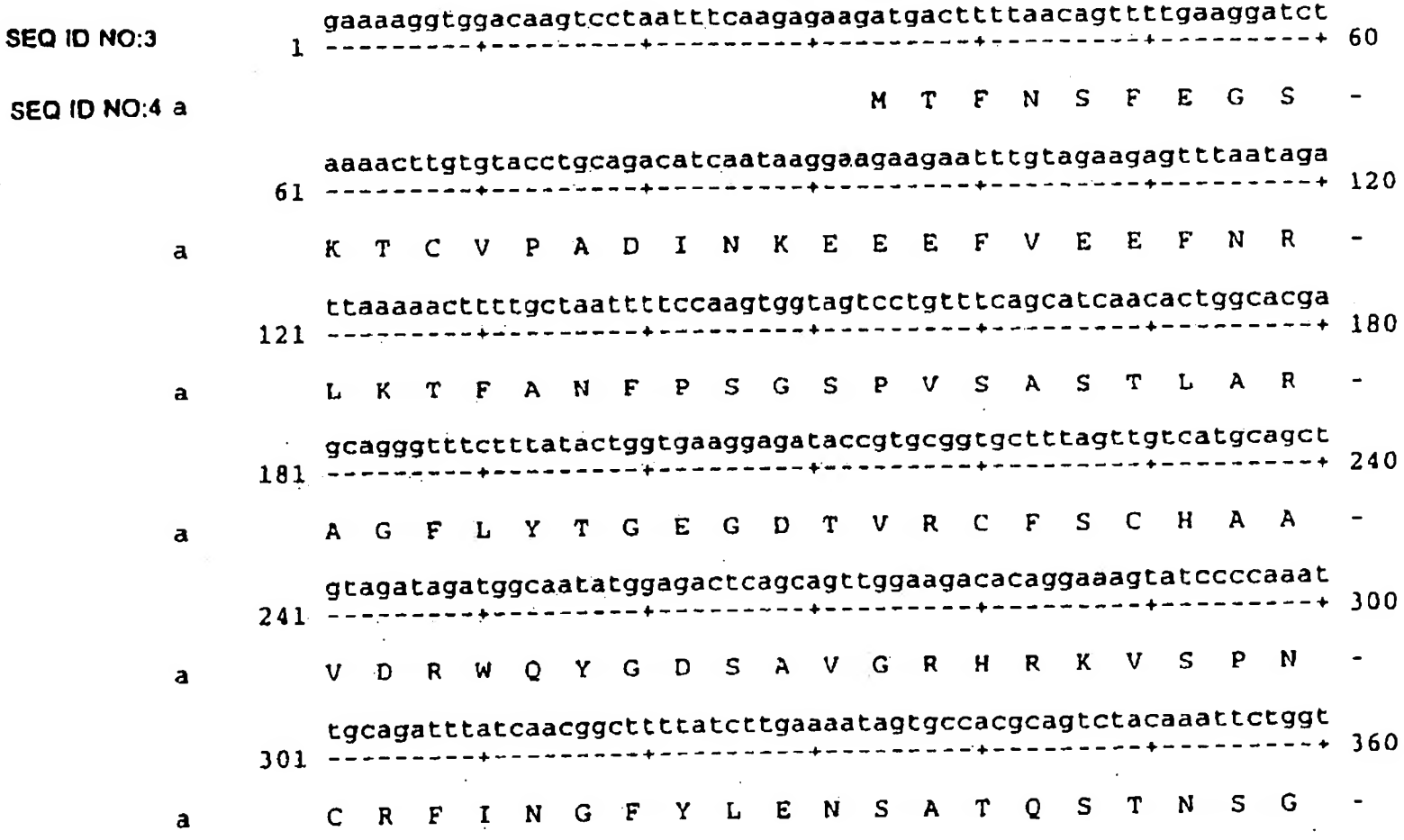

FIG. 1 (PAGE 1 OF 7)

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14.01.97

HUMAN xiap

```
      atccagaatggtcagtacaaagttgaaaactatctgggaagcagagatcattttgcctta
  361 ---------+---------+---------+---------+---------+---------+ 420
a         I  Q  N  G  Q  Y  K  V  E  N  Y  L  G  S  R  D  H  F  A  L  -

      gacaggccatctgagacacatgcagactatcttttgagaactgggcaggttgtagatata
  421 ---------+---------+---------+---------+---------+---------+ 480
a         D  R  P  S  E  T  H  A  D  Y  L  L  R  T  G  Q  V  V  D  I  -

      tcagacaccatatacccgaggaaccctgccatgtattgtgaagaagctagattaaagtcc
  481 ---------+---------+---------+---------+---------+---------+ 540
a         S  D  T  I  Y  P  R  N  P  A  M  Y  C  E  E  A  R  L  K  S  -

      tttcagaactggccagactatgctcacctaaccccaagagagttagcaagtgctggactc
  541 ---------+---------+---------+---------+---------+---------+ 600
a         F  Q  N  W  P  D  Y  A  H  L  T  P  R  E  L  A  S  A  G  L  -

      tactacacaggtattggtgaccaagtgcagtgcttttgttgtggtggaaaactgaaaaat
  601 ---------+---------+---------+---------+---------+---------+ 660
a         Y  Y  T  G  I  G  D  Q  V  Q  C  F  C  C  G  G  K  L  K  N  -

      tgggaaccttgtgatcgtgcctggtcagaacacaggcgacactttcctaattgcttcttt
  661 ---------+---------+---------+---------+---------+---------+ 720
a         W  E  P  C  D  R  A  W  S  E  H  R  R  H  F  P  N  C  F  F  -
```

FIG. 1 (PAGE 2 OF 7)

HUMAN xiap

```
     gttttgggccggaatcttaatattcgaagtgaatctgatgctgtgagttctgataggaat
721  ---------+---------+---------+---------+---------+---------+ 780
a        V  L  G  R  N  L  N  I  R  S  E  S  D  A  V  S  S  D  R  N  -

     ttcccaaattcaacaaatcttccaagaaatccatccatggcagattatgaagcacggatc
781  ---------+---------+---------+---------+---------+---------+ 840
a        F  P  N  S  T  N  L  P  R  N  P  S  M  A  D  Y  E  A  R  I  -

     tttacttttgggacatggatactcagttaacaaggagcagcttgcaagagctggattt
841  ---------+---------+---------+---------+---------+---------+ 900
a        F  T  F  G  T  W  I  Y  S  V  N  K  E  Q  L  A  R  A  G  F  -

     tatgctttaggtgaaggtgataaagtaaagtgctttcactgtggaggagggctaactgat
901  ---------+---------+---------+---------+---------+---------+ 960
a        Y  A  L  G  E  G  D  K  V  K  C  F  H  C  G  G  G  L  T  D  -

     tggaagcccagtgaagacccttgggaacaacatgctaaatggtatccagggtgcaaatat
961  ---------+---------+---------+---------+---------+---------+ 1020
a        W  K  P  S  E  D  P  W  E  Q  H  A  K  W  Y  P  G  C  K  Y  -

     ctgttagaacagaagggacaagaatatataaacaatattcatttaactcattcacttgag
1021 ---------+---------+---------+---------+---------+---------+ 1080
a        L  L  E  Q  K  G  Q  E  Y  I  N  N  I  H  L  T  H  S  L  E  -
```

FIG. 1 (PAGE 3 OF 7)

HUMAN xiap

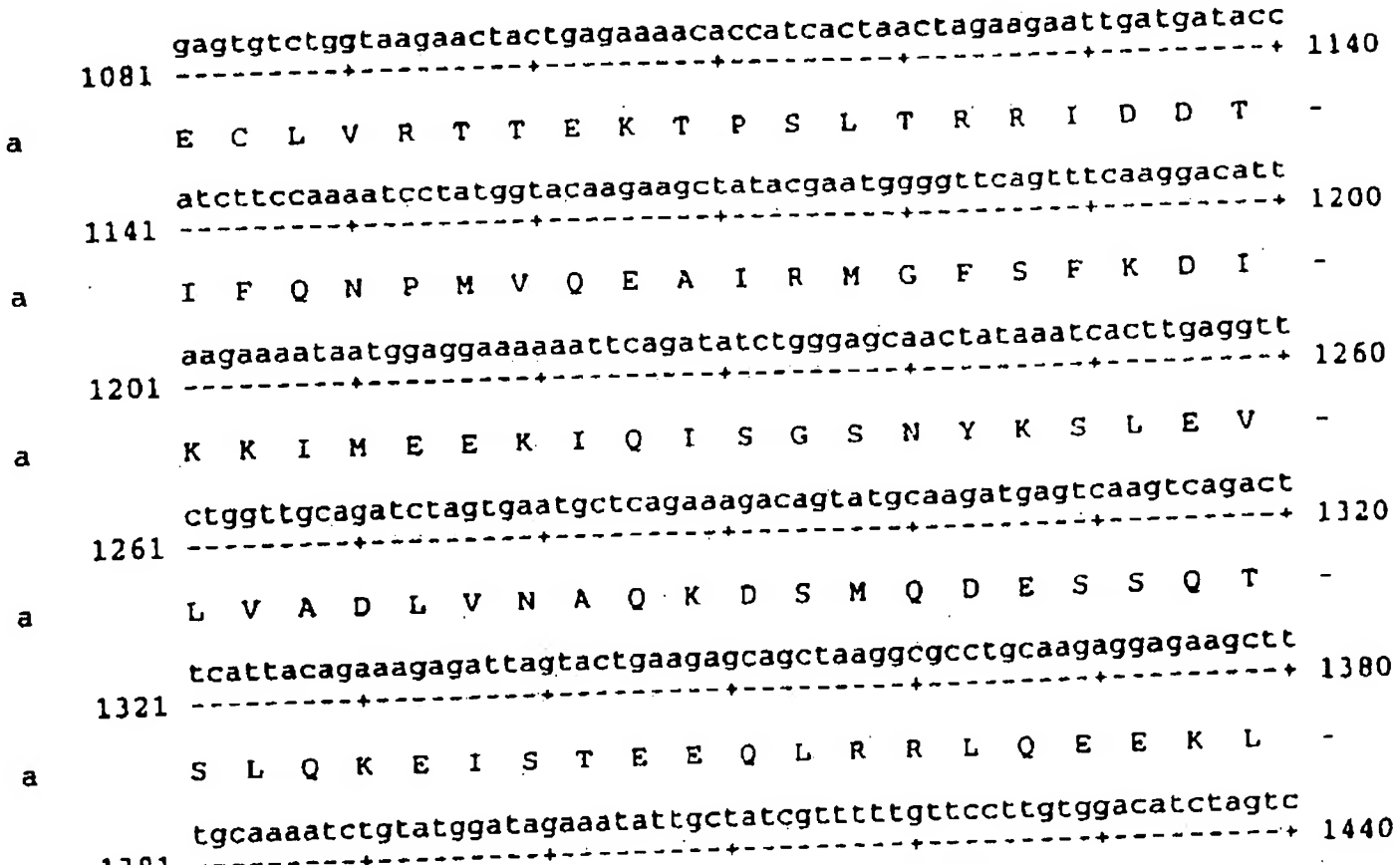

FIG. 1 (PAGE 4 OF 7)

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14.01.97

HUMAN xiap

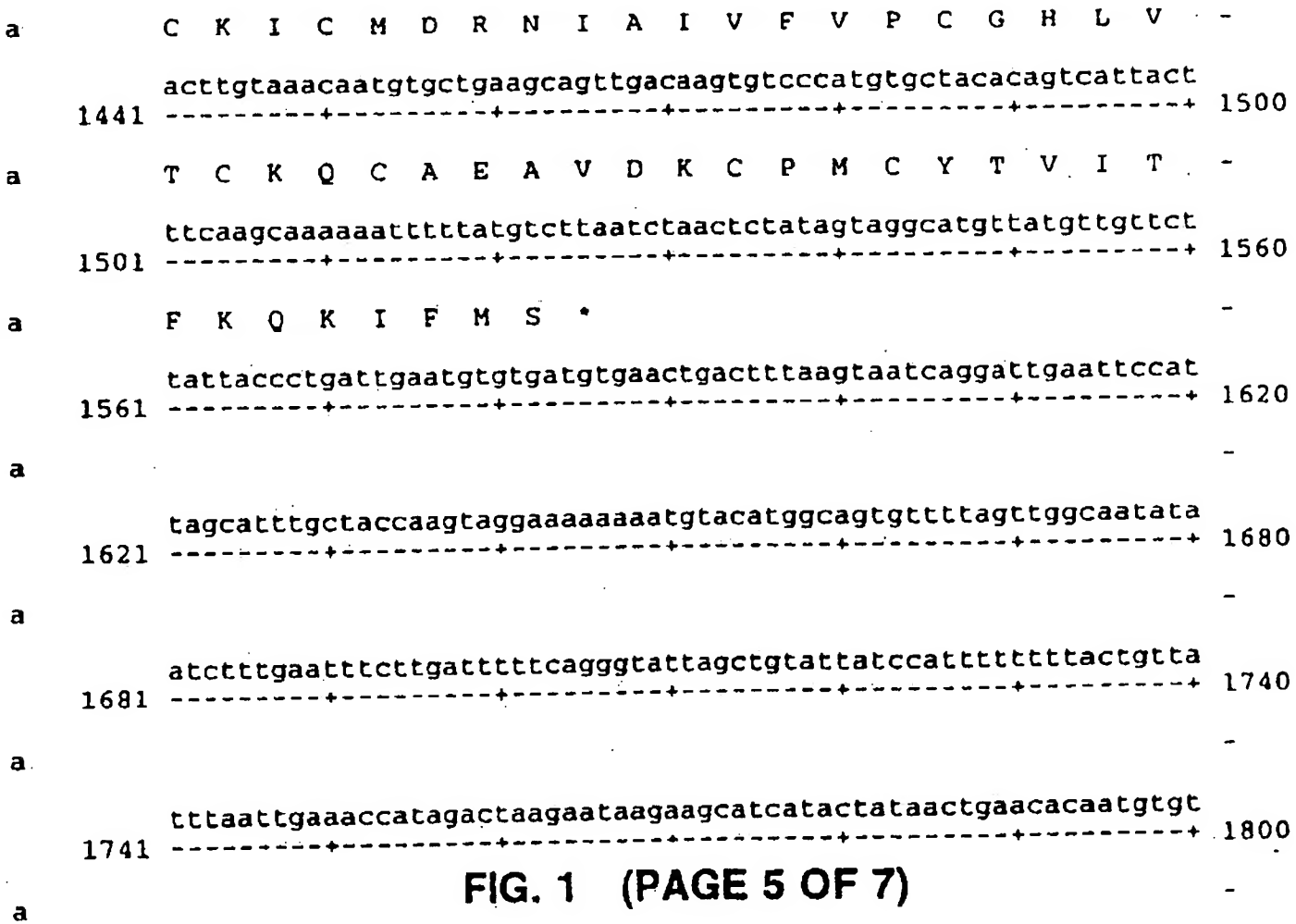

FIG. 1 (PAGE 5 OF 7)

HUMAN xiap

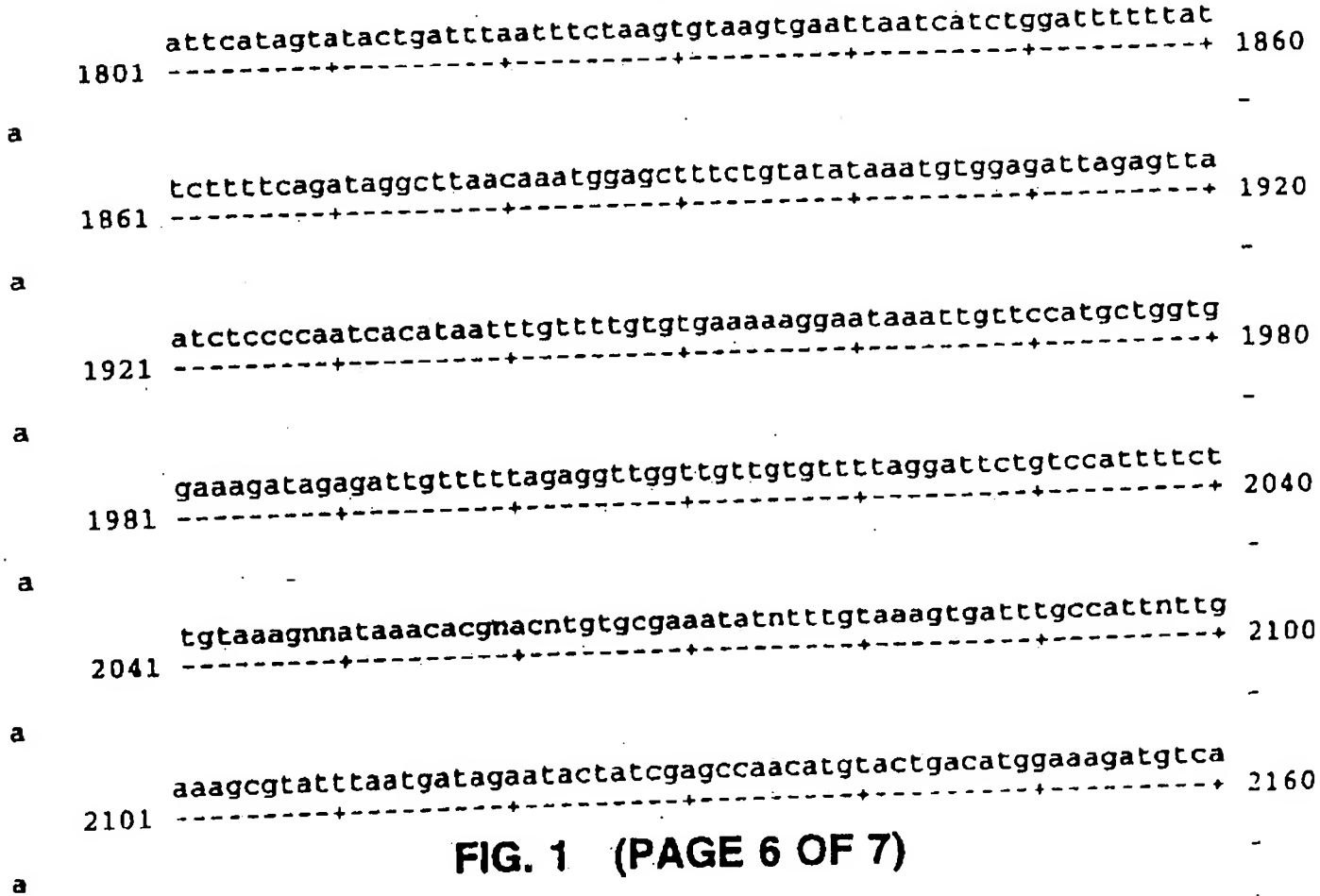

FIG. 1 (PAGE 6 OF 7)

PCT/IB 96/01022
14.01.97

HUMAN xiap

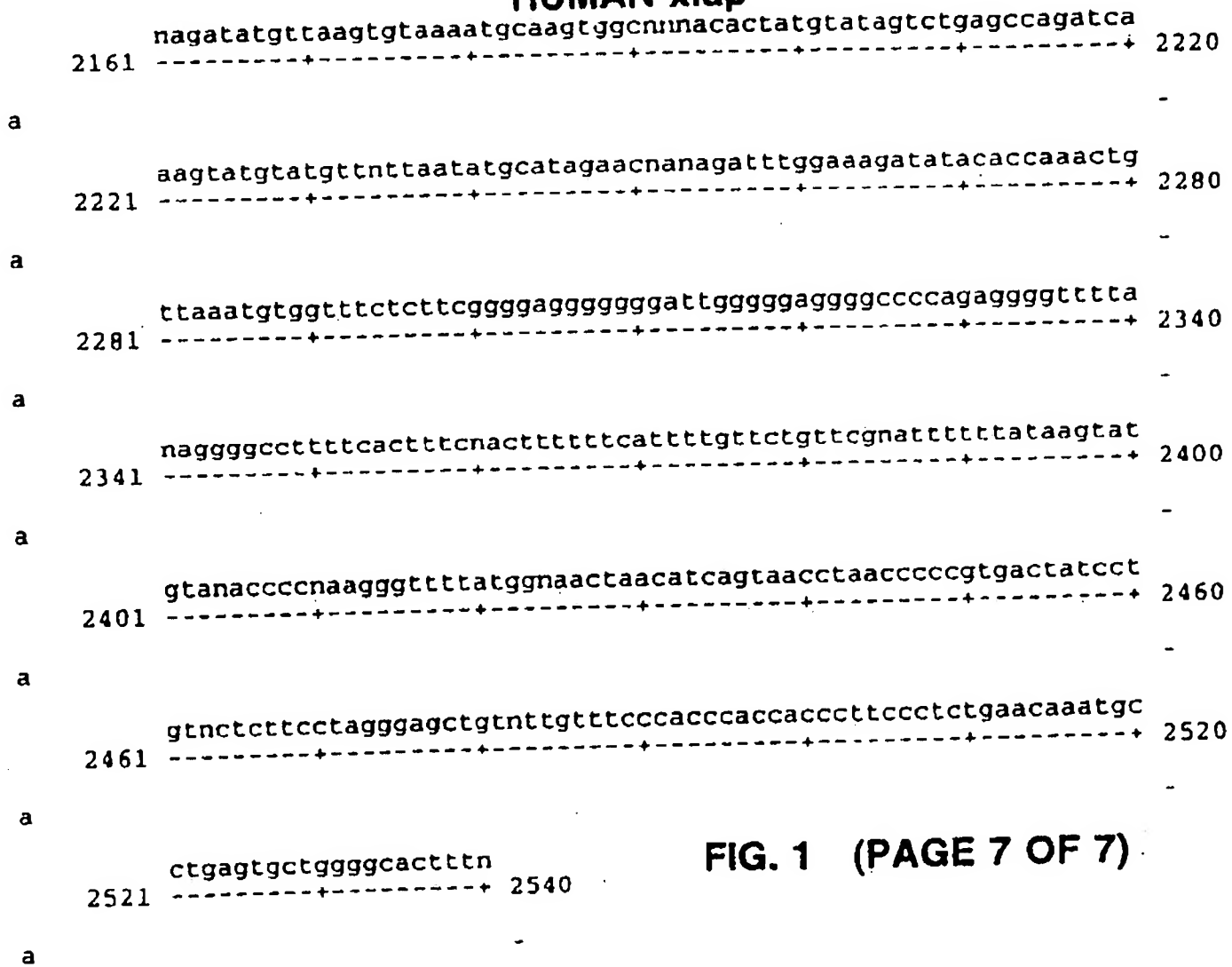

FIG. 1 (PAGE 7 OF 7)

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HUMAN hiap-1

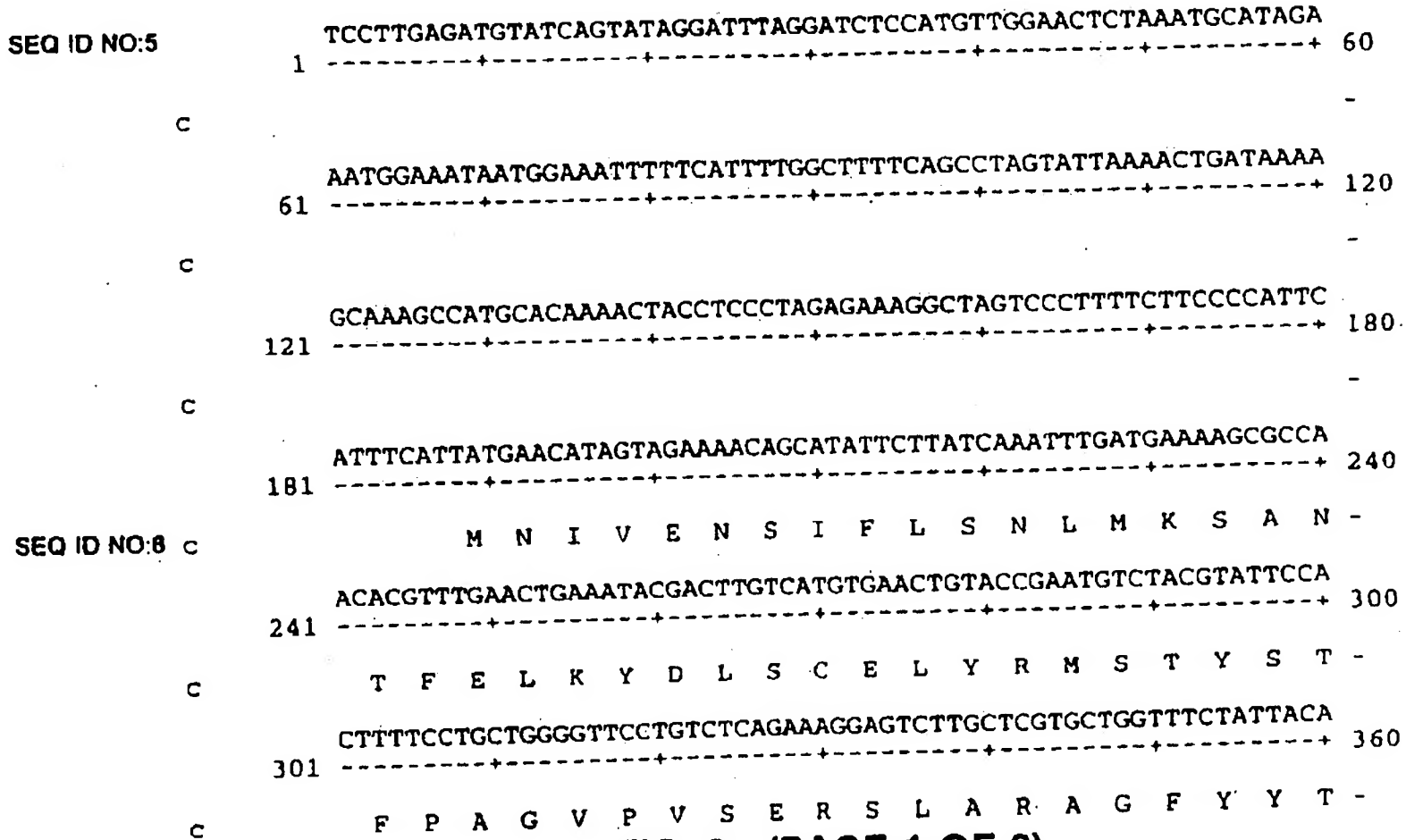

FIG. 2 (PAGE 1 OF 8)

HUMAN hiap-1

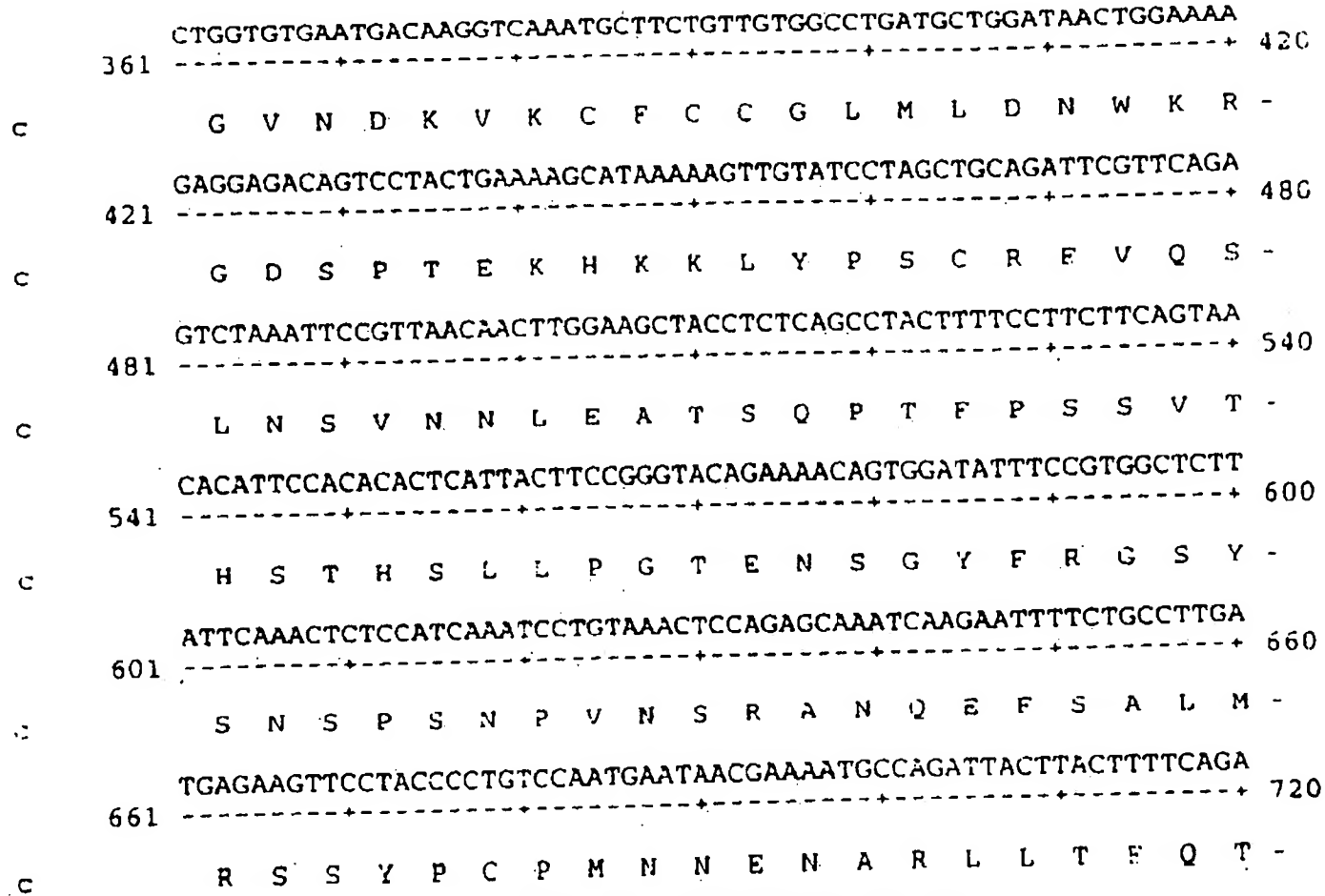

FIG. 2 (PAGE 2 OF 8)

HUMAN hiap-1

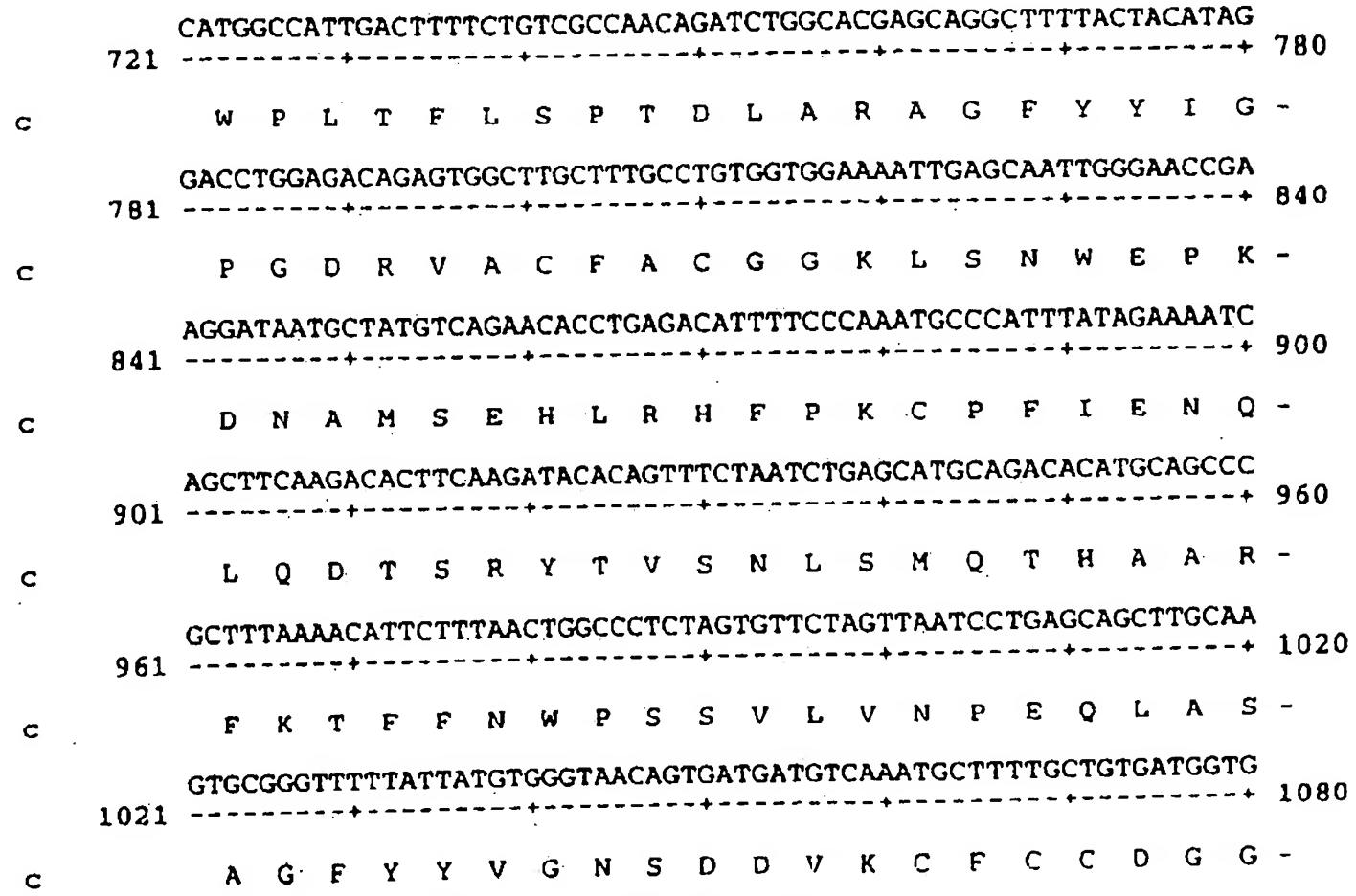

FIG. 2 (PAGE 3 OF 8)

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HUMAN hiap-1

```
     GACTCAGGTGTTGGGAATCTGGAGATGATCCATGGGTTCAACATGCCAAGTGGTTTCCAA
1081 ---------+---------+---------+---------+---------+---------+ 1140

C          L  R  C  W  E  S  G  D  D  P  W  V  Q  H  A  K  W  F  P  R -

     GGTGTGAGTACTTGATAAGAATTAAAGGACAGGAGTTCATCCGTCAAGTTCAAGCCAGTT
1141 ---------+---------+---------+---------+---------+---------+ 1200

C          C  E  Y  L  I  R  I  K  G  Q  E  F  I  R  Q  V  Q  A  S  Y -

     ACCCTCATCTACTTGAACAGCTGCTATCCACATCAGACAGCCCAGGAGATGAAAATGCAG
1201 ---------+---------+---------+---------+---------+---------+ 1260

C          P  H  L  L  E  Q  L  L  S  T  S  D  S  P  G  D  E  N  A  E -

     AGTCATCAATTATCCATTTGGAACCTGGAGAAGACCATTCAGAAGATGCAATCATGATGA
1261 ---------+---------+---------+---------+---------+---------+ 1320

C          S  S  I  I  H  L  E  P  G  E  D  H  S  E  D  A  I  M  M  N -

     ATACTCCTGTGATTAATGCTGCCGTGGAAATGGGCTTTAGTAGAAGCCTGGTAAAACAGA
1321 ---------+---------+---------+---------+---------+---------+ 1380

C          T  P  V  I  N  A  A  V  E  M  G  F  S  R  S  L  V  K  Q  T -

     CAGTTCAGAGAAAAATCCTAGCAACTGGAGAGAATTATAGACTAGTCAATGATCTTGTGT
1381 ---------+---------+---------+---------+---------+---------+ 1440

C          V  Q  R  K  I  L  A  T  G  E  N  Y  R  L  V  N  D  L  V  L -
```

FIG. 2 (PAGE 4 OF 8)

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HUMAN hiap-1

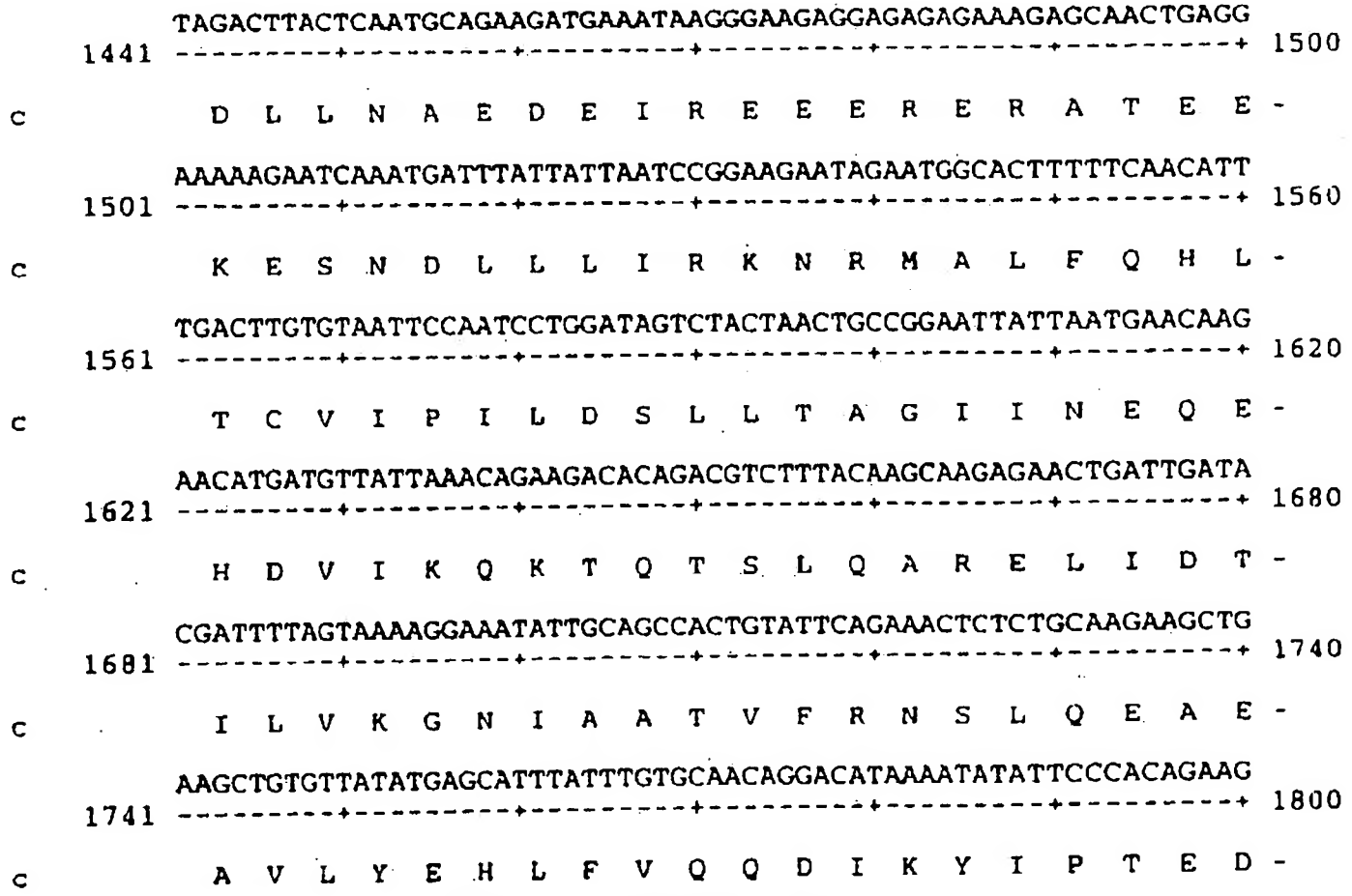

FIG. 2 (PAGE 5 OF 8)

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HUMAN hiap-1

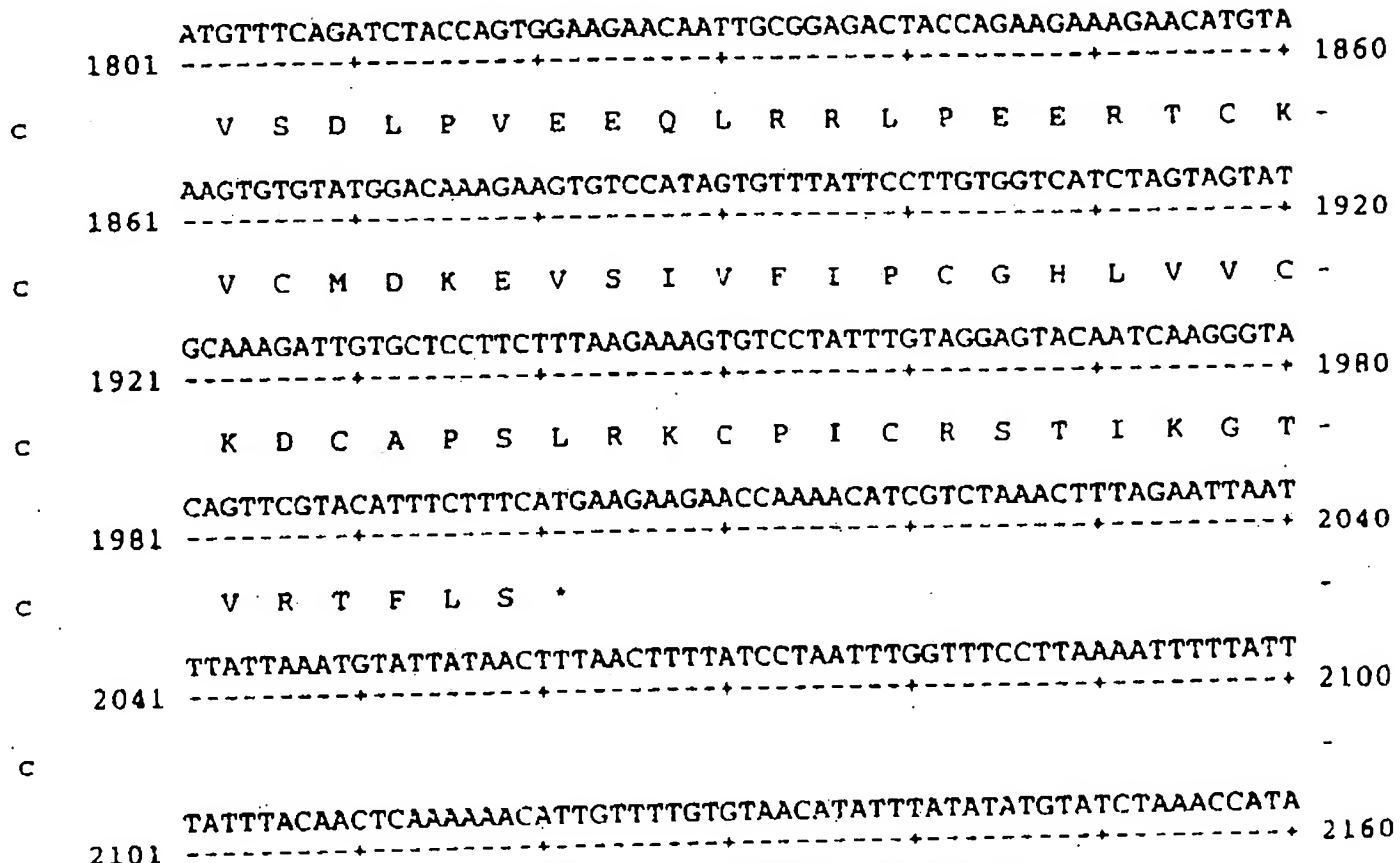

FIG. 2 (PAGE 6 OF 8)

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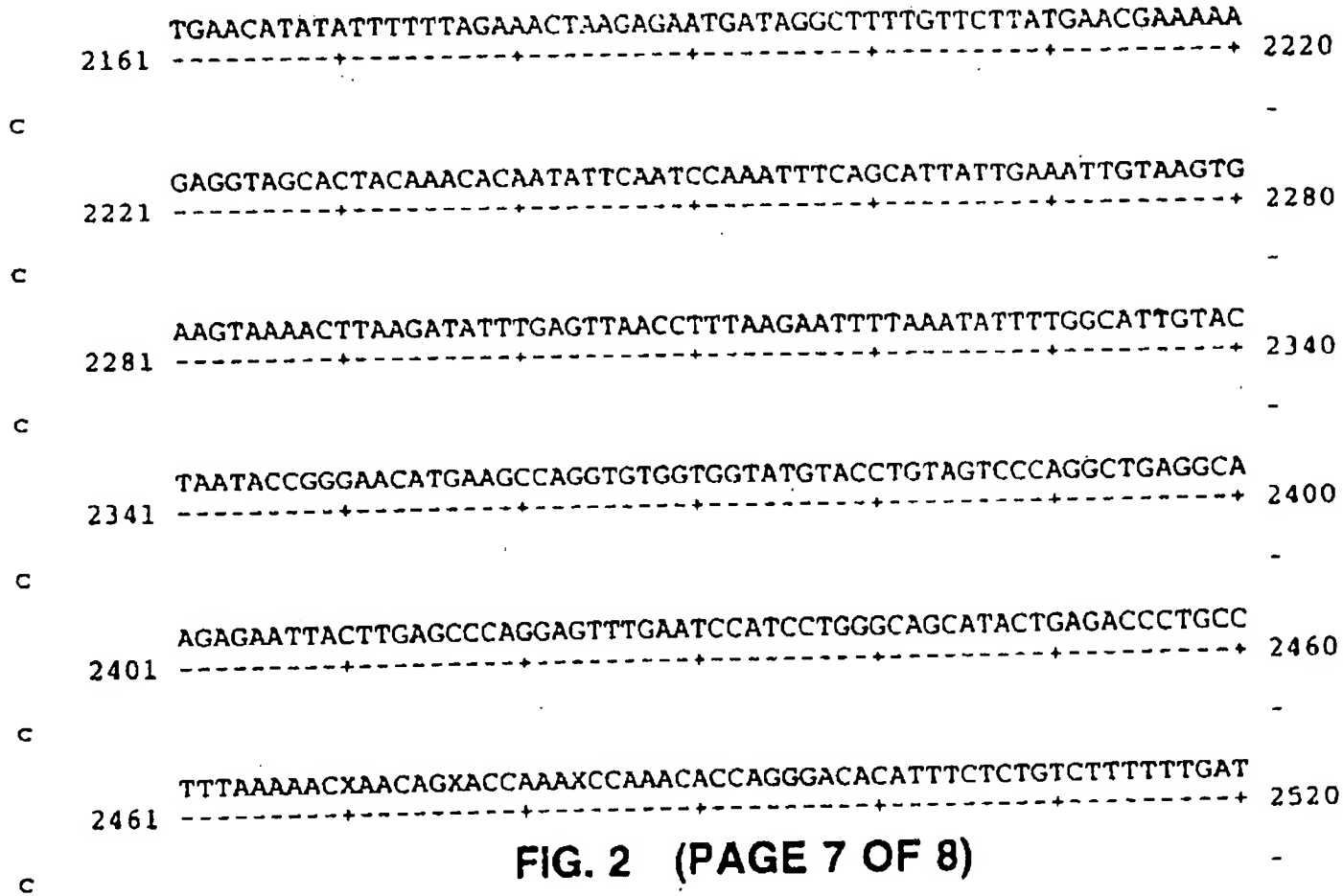
HUMAN hiap-1



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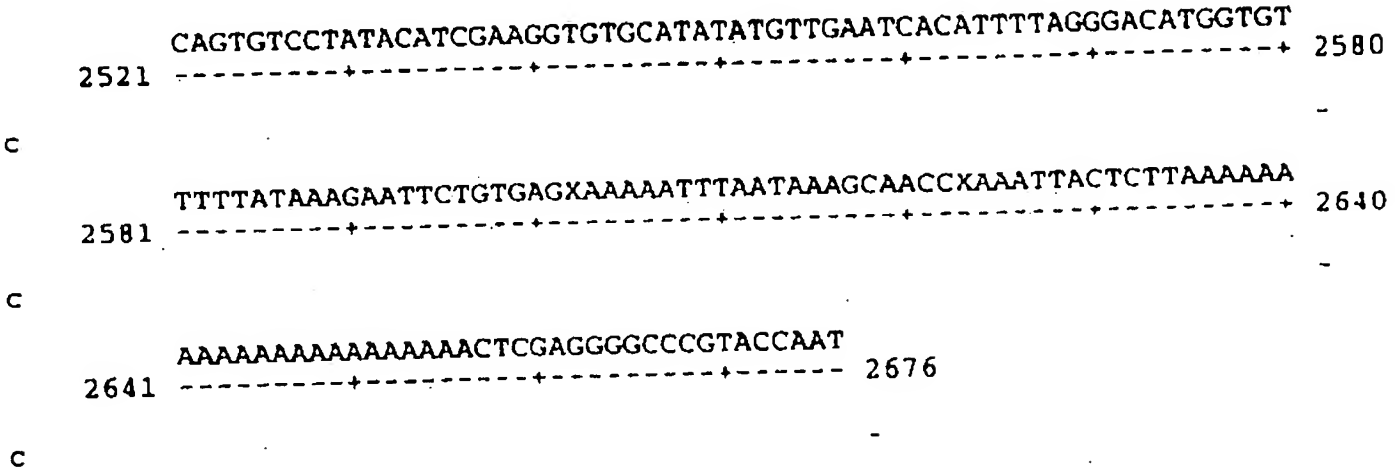
HUMAN hiap-1



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HUMAN hiap-2

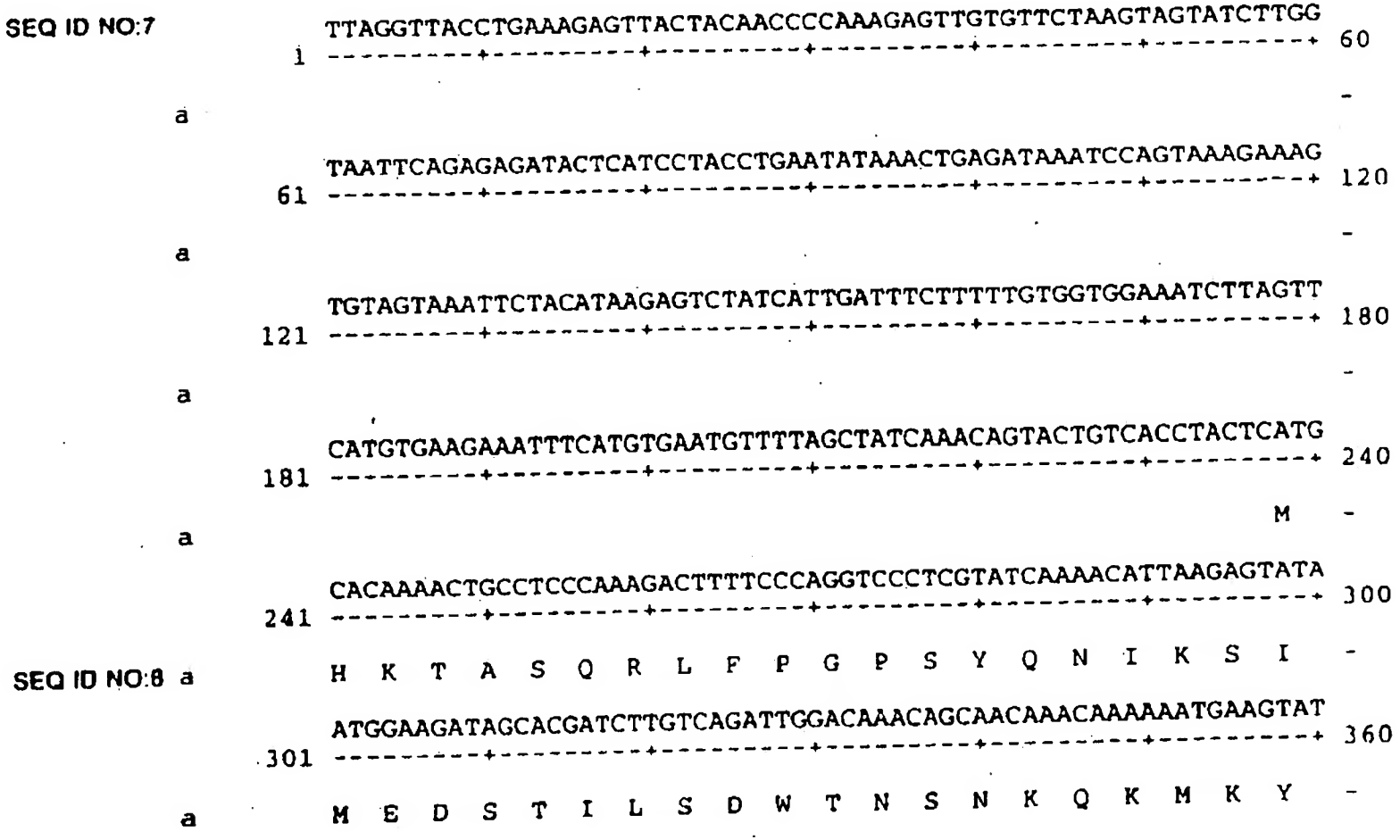

FIG. 3 (PAGE 1 OF 7)

HUMAN hiap-2

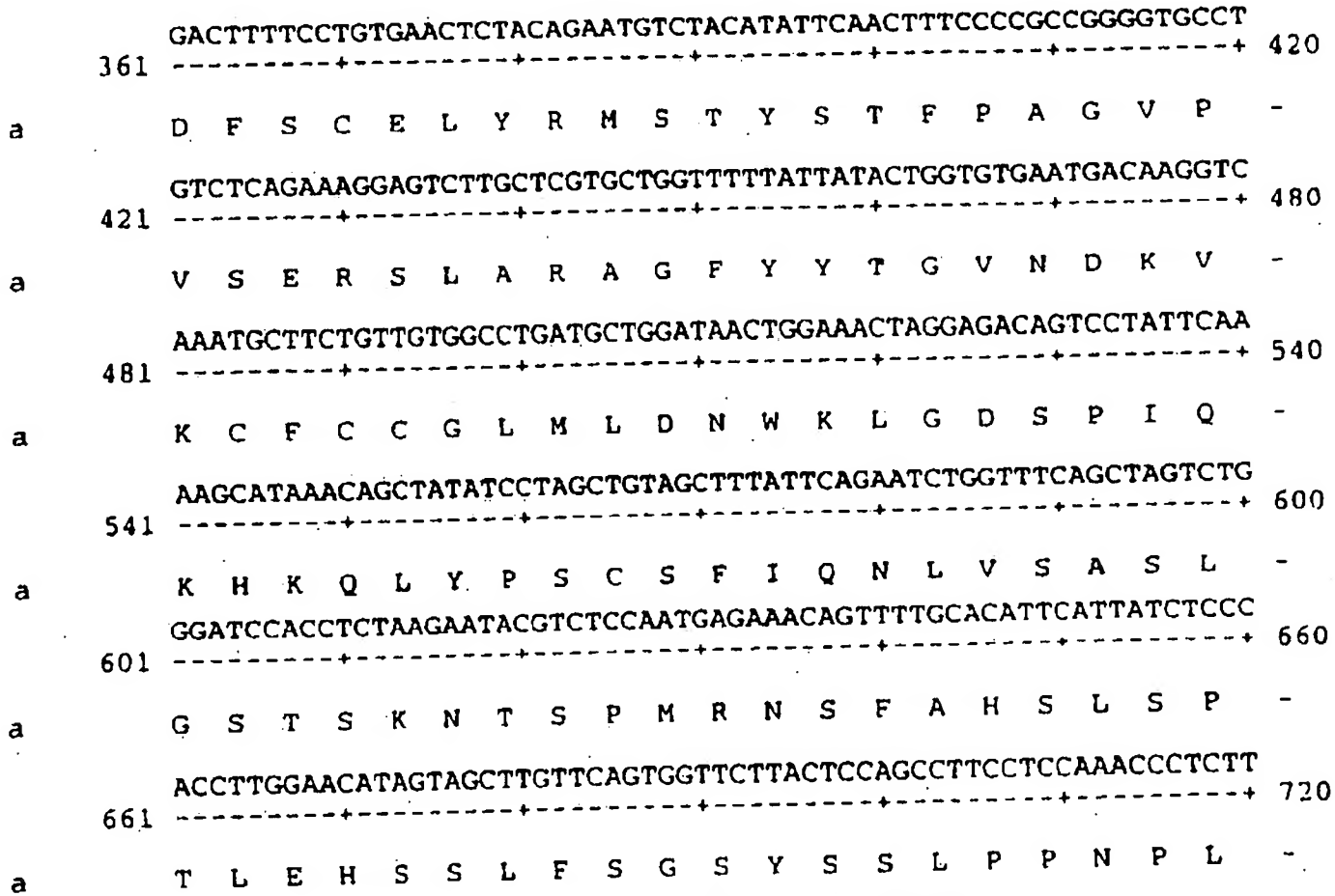

FIG. 3 (PAGE 2 OF 7)

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HUMAN hiap-2

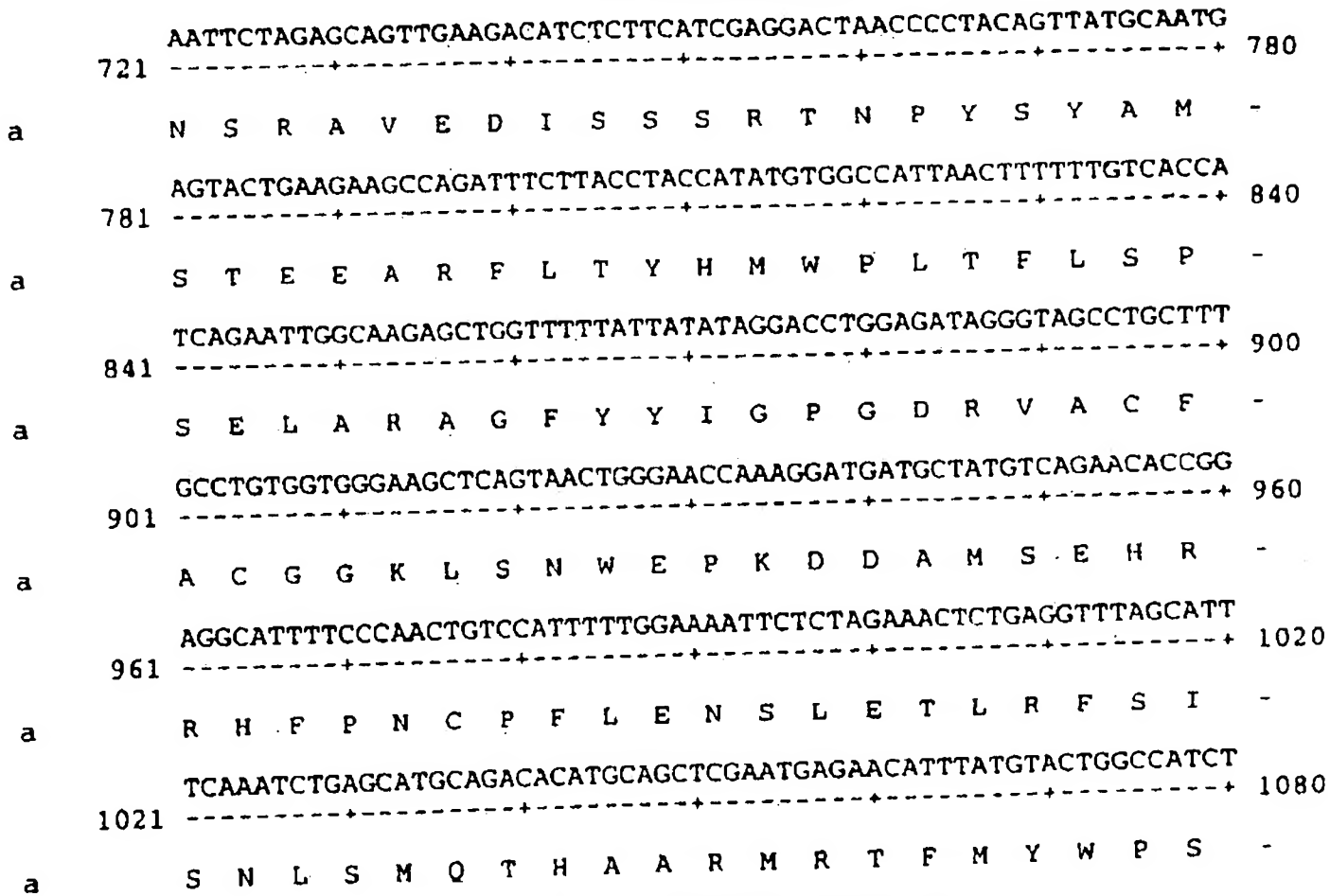

FIG. 3 (PAGE 3 OF 7)

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HUMAN hiap-2

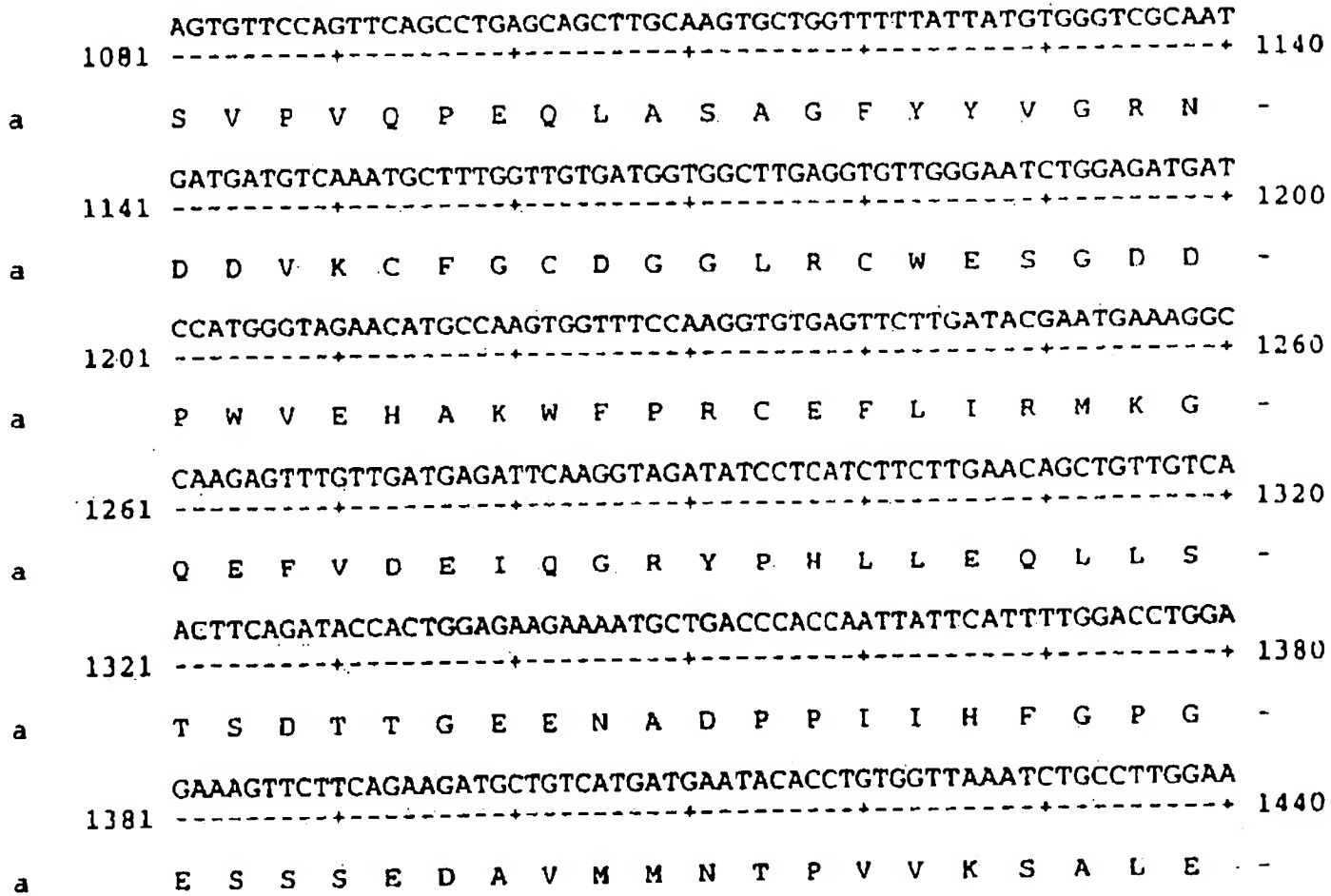

FIG. 3 (PAGE 4 OF 7)

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HUMAN hiap-2

```
     ATGGGCTTTAATAGAGACCTGGTGAAACAAACAGTTCTAAGTAAAATCCTGACAACTGGA
1441 ---------+---------+---------+---------+---------+---------+ 1500

a    M  G  F  N  R  D  L  V  K  Q  T  V  L  S  K  I  L  T  T  G  -

     GAGAACTATAAAACAGTTAATGATATTGTGTCAGCACTTCTTAATGCTGAAGATGAAAAA
1501 ---------+---------+---------+---------+---------+---------+ 1560

a    E  N  Y  K  T  V  N  D  I  V  S  A  L  L  N  A  E  D  E  K  -

     AGAGAAGAGGAGAAGGAAAAACAAGCTGAAGAAATGGCATCAGATGATTTGTCATTAATT
1561 ---------+---------+---------+---------+---------+---------+ 1620

a    R  E  E  E  K  E  K  Q  A  E  E  M  A  S  D  D  L  S  L  I  -

     CGGAAGAACAGAATGGCTCTCTTTCAACAATTGACATGTGTGCTTCCTATCCTGGATAAT
1621 ---------+---------+---------+---------+---------+---------+ 1680

a    R  K  N  R  M  A  L  F  Q  Q  L  T  C  V  L  P  I  L  D  N  -

     CTTTTAAAGGCCAATGTAATTAATAAACAGGAACATGATATTATTAAACAAAAAACACAG
1681 ---------+---------+---------+---------+---------+---------+ 1740

a    L  L  K  A  N  V  I  N  K  Q  E  H  D  I  I  K  Q  K  T  Q  -

     ATACCTTTACAAGCGAGAGAACTGATTGATACCATTTGGGTTAAAGGAAATGCTGCGGCC
1741 ---------+---------+---------+---------+---------+---------+ 1800

a    I  P  L  Q  A  R  E  L  I  D  T  I  W  V  K  G  N  A  A  A  -
```

FIG. 3 (PAGE 5 OF 7)

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HUMAN hiap-2

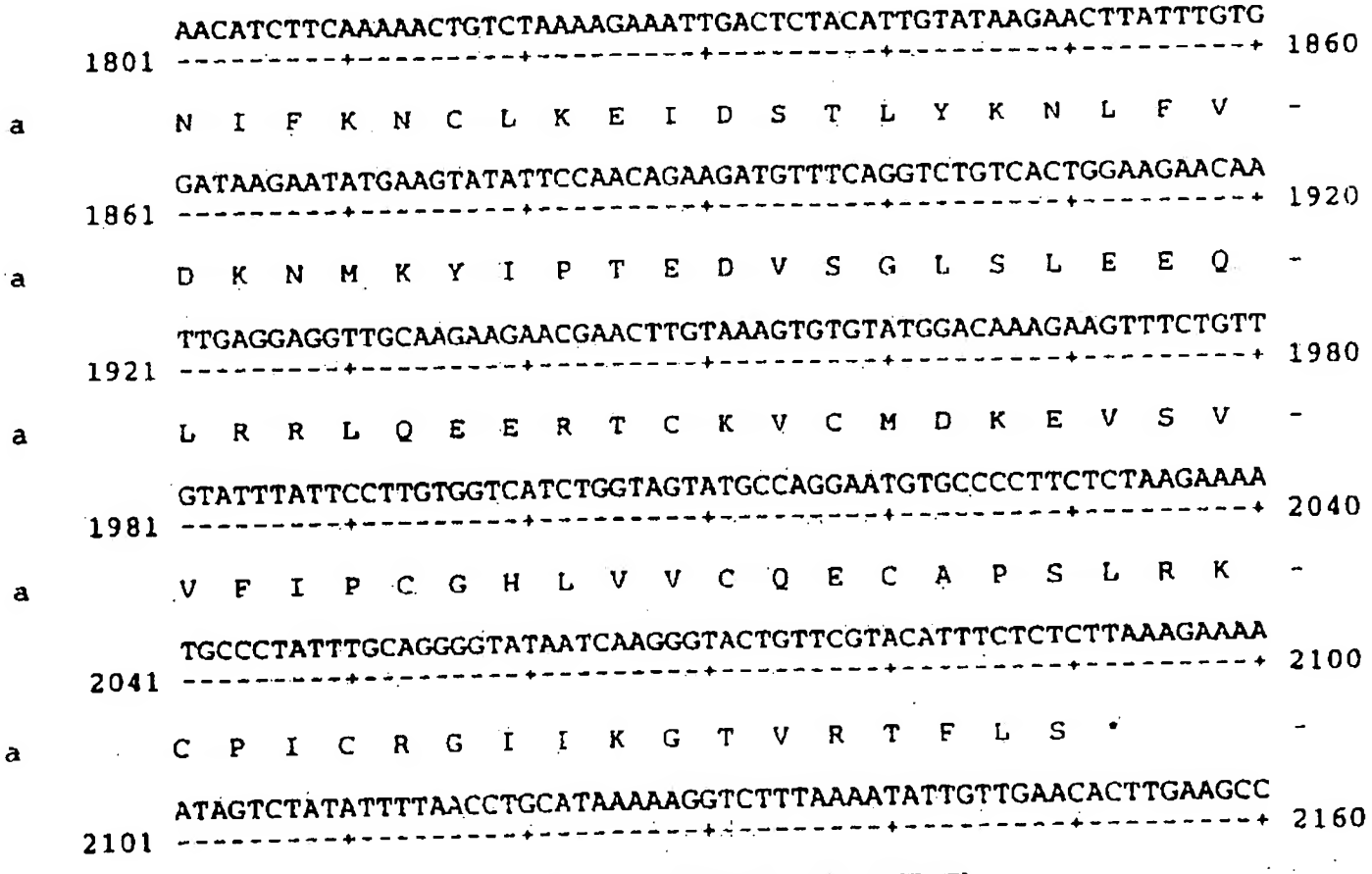

FIG. 3 (PAGE 6 OF 7)

HUMAN hiap-2

FIG. 3 (PAGE 7 OF 7)

MOUSE xiap

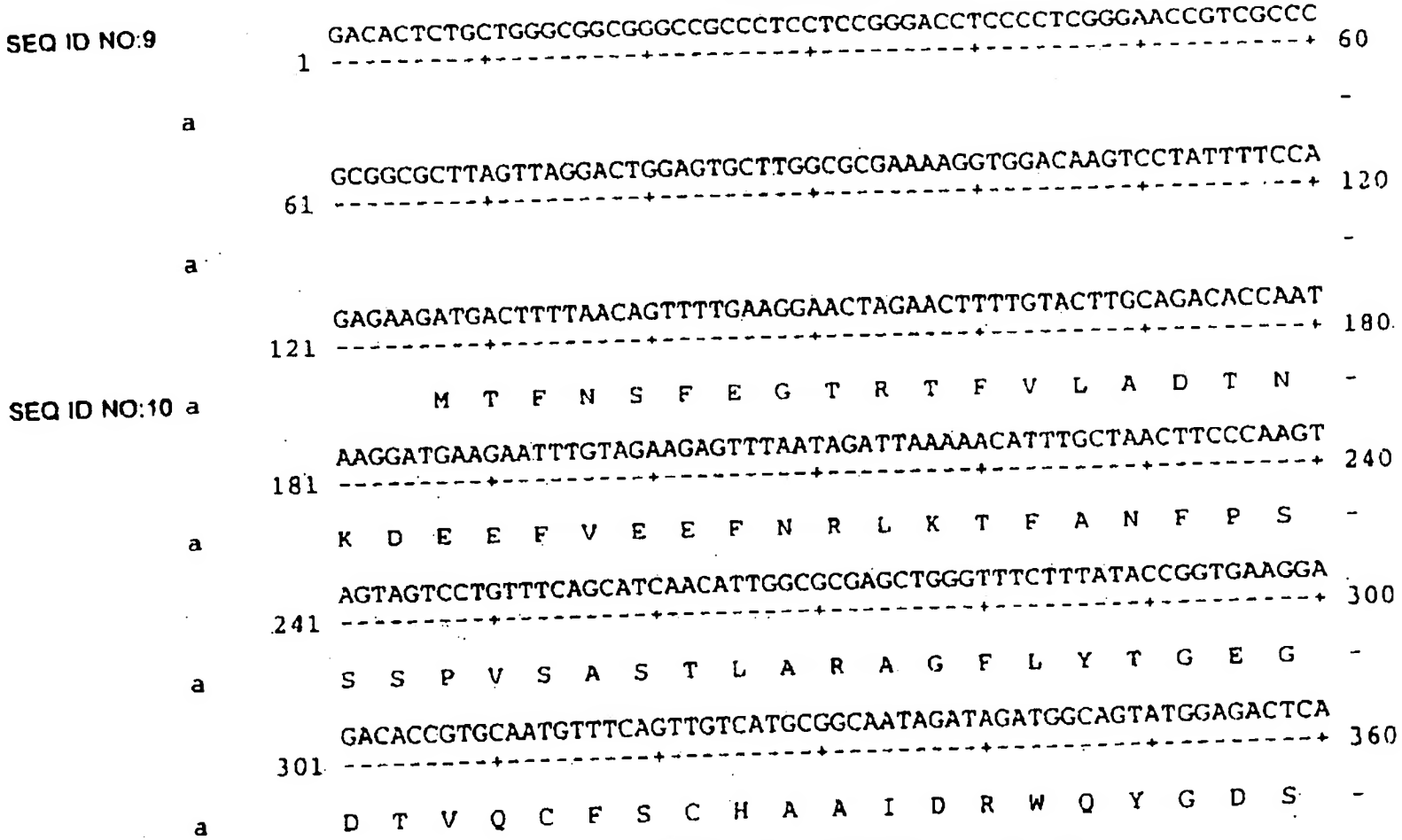

FIG. 4 (PAGE 1 OF 6)

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MOUSE xiap

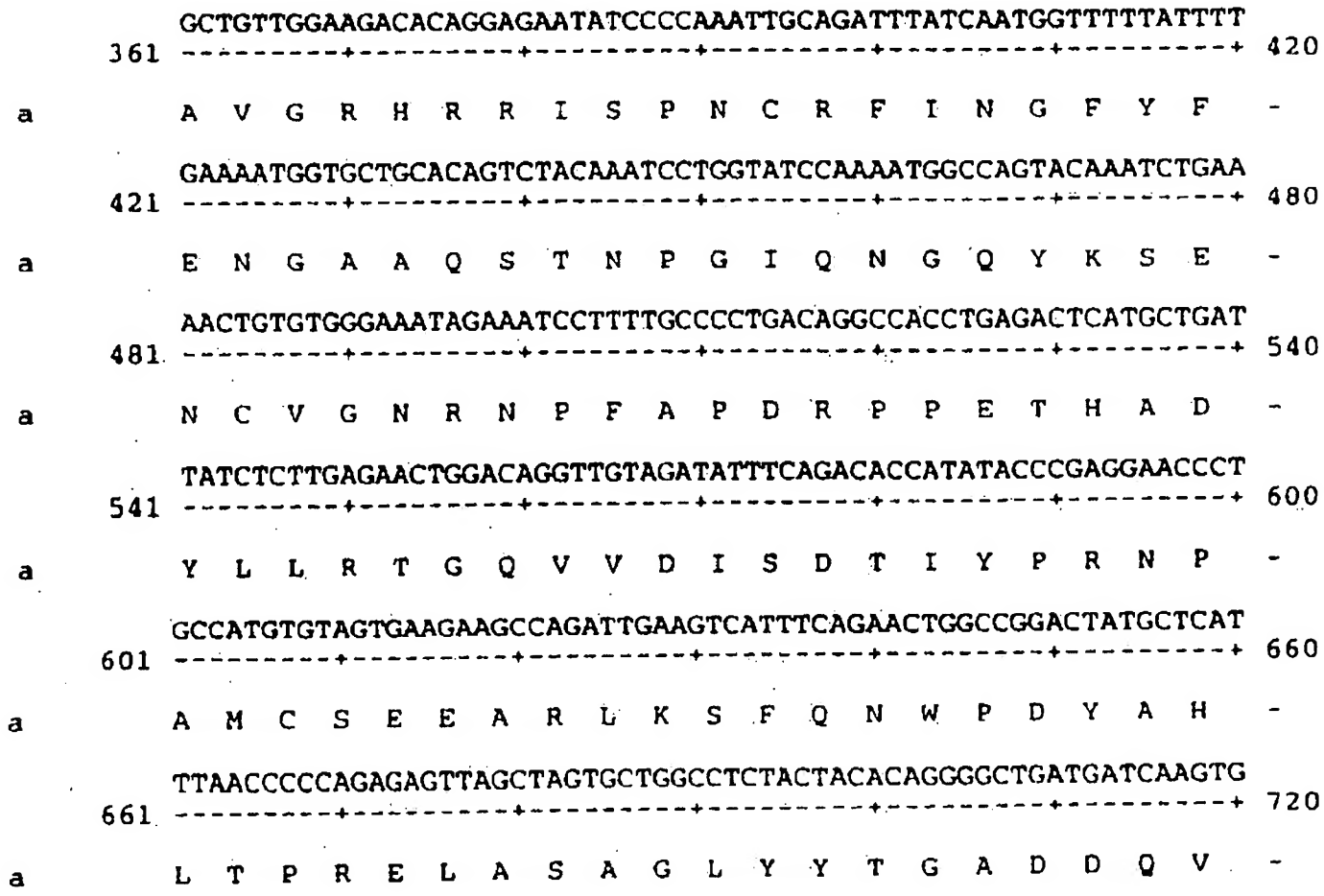

FIG. 4 (PAGE 2 OF 6)

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MOUSE xiap

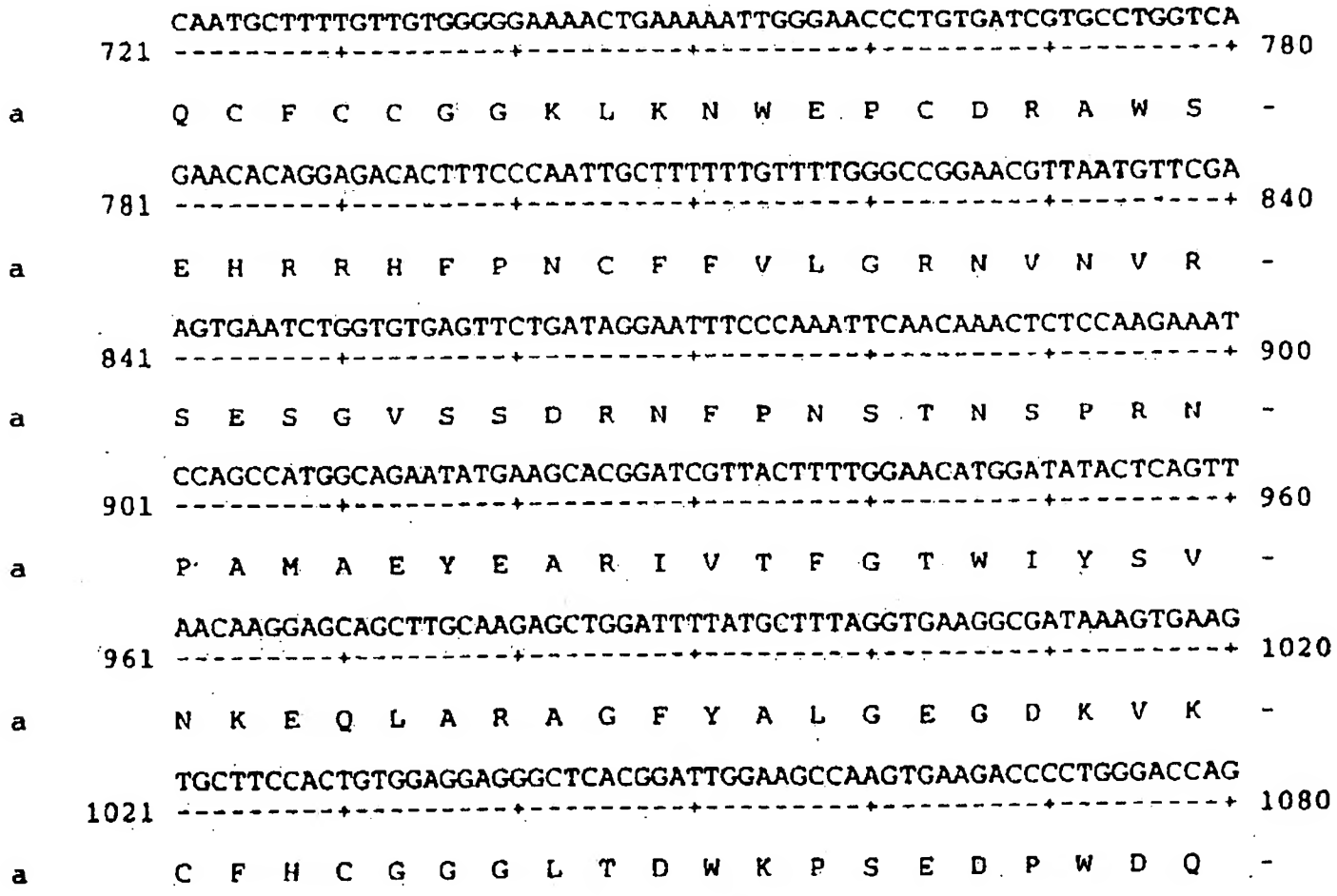

FIG. 4 (PAGE 3 OF 6)

MOUSE xiap

```
     CATGCTAAGTGCTACCCAGGGTGCAAATACCTATTGGATGAGAAGGGGCAAGAATATATA
1081 ---------+---------+---------+---------+---------+---------+ 1140

a      H  A  K  C  Y  P  G  C  K  Y  L  L  D  E  K  G  Q  E  Y  I  -

     AATAATATTCATTTAACCCATCCACTTGAGGAATCTTTGGGAAGAACTGCTGAAAAAACA
1141 ---------+---------+---------+---------+---------+---------+ 1200

a      N  N  I  H  L  T  H  P  L  E  E  S  L  G  R  T  A  E  K  T  -

     CCACCGCTAACTAAAAAAATCGATGATACCATCTTCCAGAATCCTATGGTGCAAGAAGCT
1201 ---------+---------+---------+---------+---------+---------+ 1260

a      P  P  L  T  K  K  I  D  D  T  I  F  Q  N  P  M  V  Q  E  A  -

     ATACGAATGGGATTTAGCTTCAAGGACCTTAAGAAAACAATGGAAGAAAAAATCCAAACA
1261 ---------+---------+---------+---------+---------+---------+ 1320

a      I  R  M  G  F  S  F  K  D  L  K  K  T  M  E  E  K  I  Q  T  -

     TCCGGGAGCAGCTATCTATCACTTGAGGTCCTGATTGCAGATCTTGTGAGTGCTCAGAAA
1321 ---------+---------+---------+---------+---------+---------+ 1380

a      S  G  S  S  Y  L  S  L  E  V  L  I  A  D  L  V  S  A  Q  K  -

     GATAATACGGAGGATGAGTCAAGTCAAACTTCATTGCAGAAAGACATTAGTACTGAAGAG
1381 ---------+---------+---------+---------+---------+---------+ 1440

a      D  N  T  E  D  E  S  S  Q  T  S  L  Q  K  D  I  S  T  E  E  -
```

FIG. 4 (PAGE 4 OF 6)

MOUSE xiap

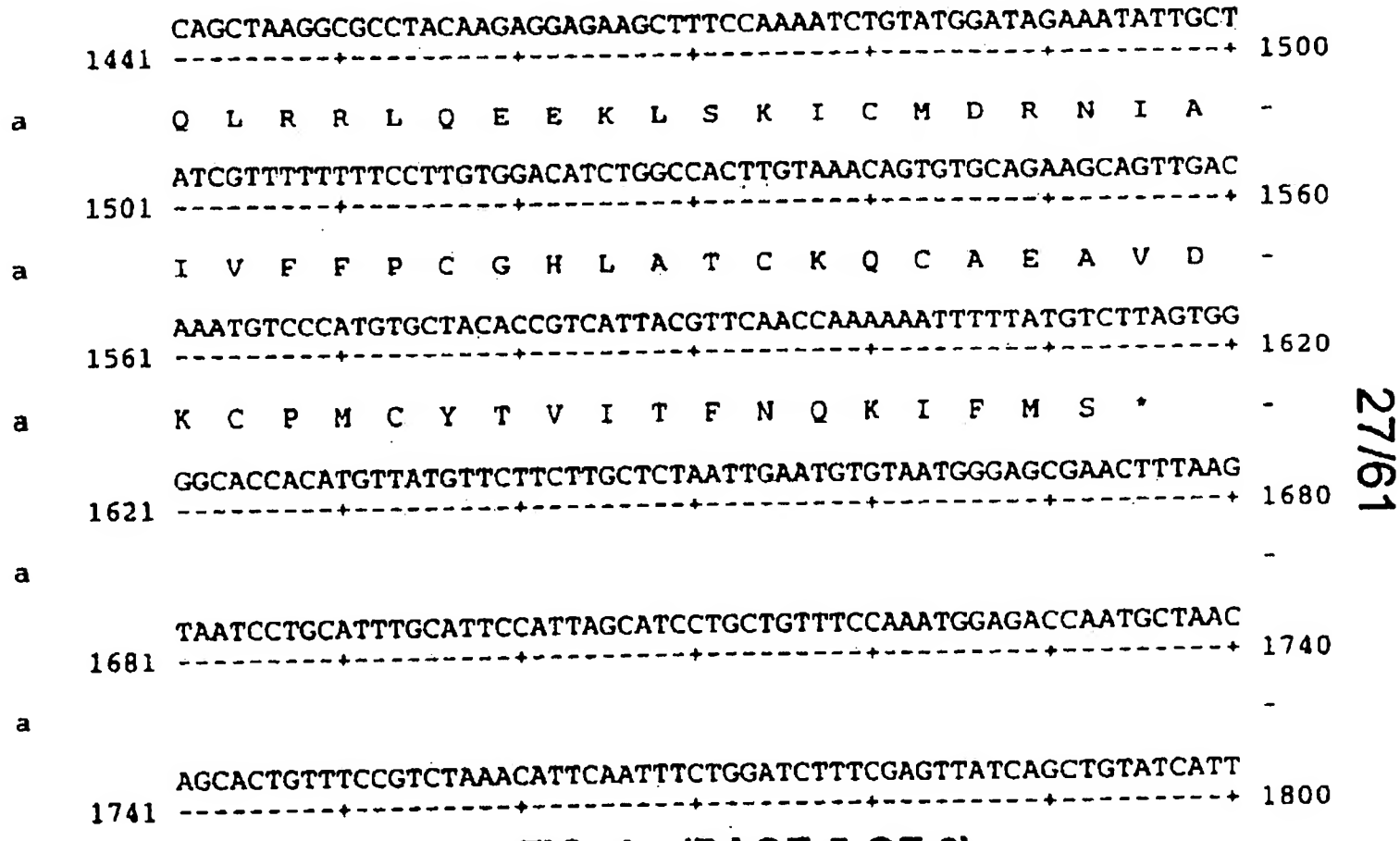

FIG. 4 (PAGE 5 OF 6)

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14.01.97

MOUSE xiap

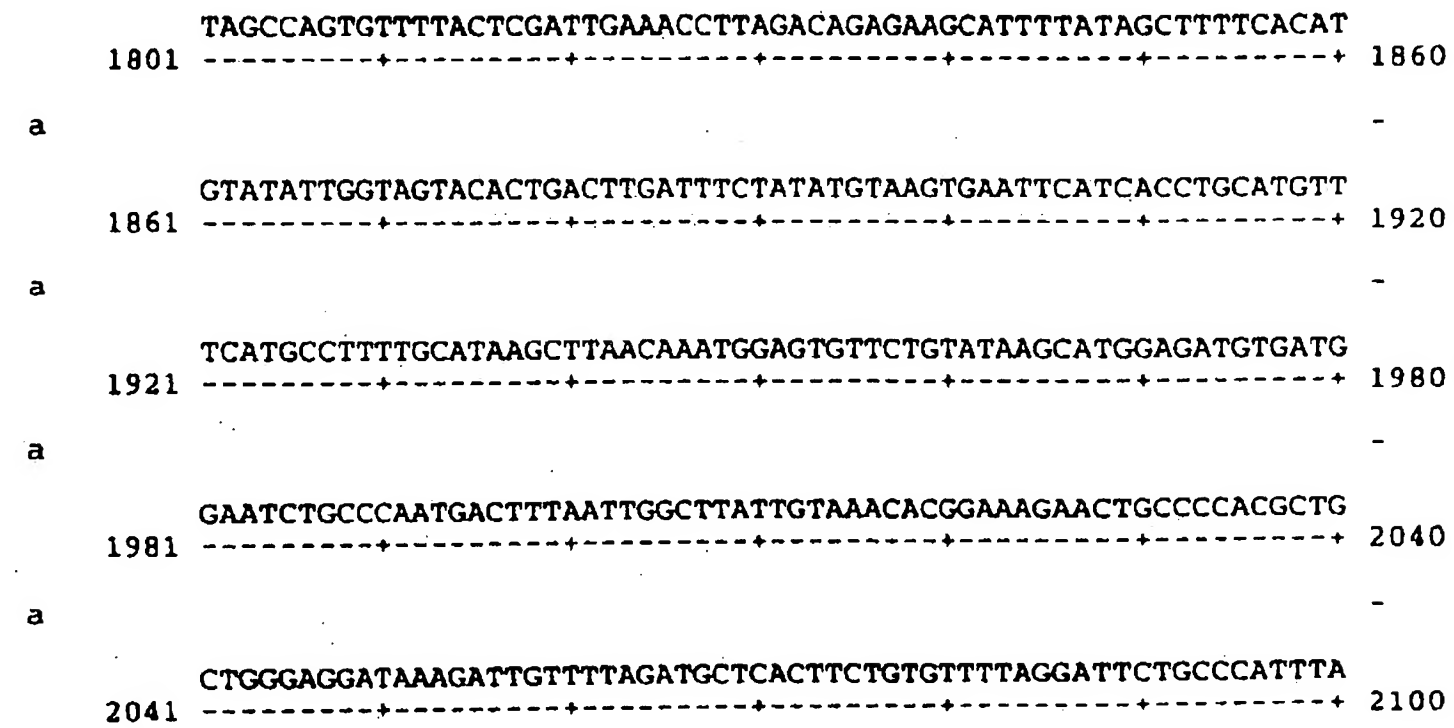

FIG. 4 (PAGE 6 OF 6)

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14.01.97

M-hiap-1

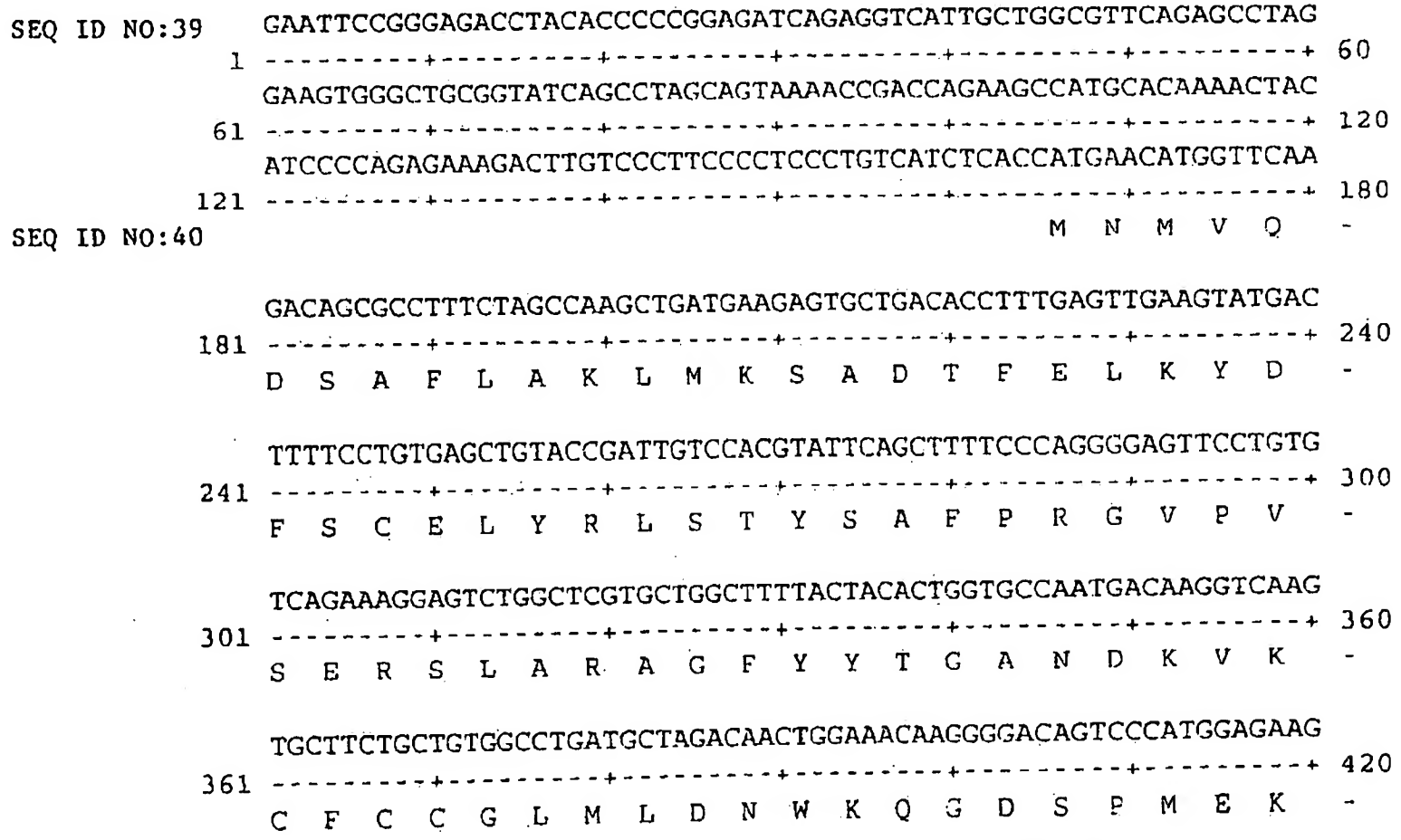

FIG. 5 (PAGE 1 OF 6)

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M-hiap-1

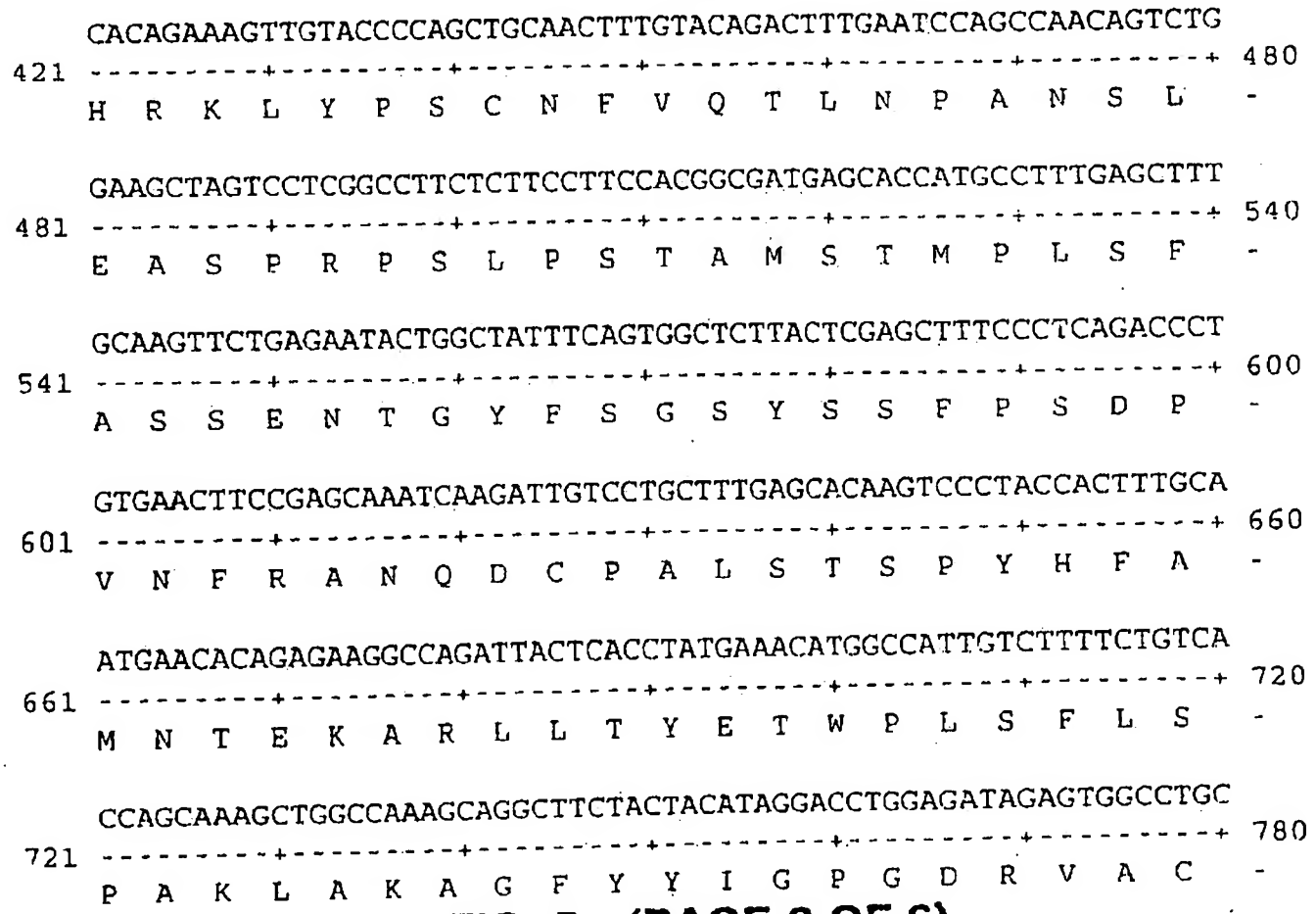

FIG. 5 (PAGE 2 OF 6)

PCT/IB 96/01022
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M-hiap-1

```
    TTTGCGTGCGATGGGAAACTGAGCAACTGGGAACGTAAGGATGATGCTATGTCAGAGCAC
781 ---------+---------+---------+---------+---------+---------+ 840
    F  A  C  D  G  K  L  S  N  W  E  R  K  D  D  A  M  S  E  H  -

    CAGAGGCATTTCCCCAGCTGTCCGTTCTTAAAAGACTTGGGTCAGTCTGCTTCGAGATAC
841 ---------+---------+---------+---------+---------+---------+ 900
    Q  R  H  F  P  S  C  P  F  L  K  D  L  G  Q  S  A  S  R  Y  -

    ACTGTCTCTAACCTGAGCATGCAGACACACGCAGCCCGTATTAGAACATTCTCTAACTGG
901 ---------+---------+---------+---------+---------+---------+ 960
    T  V  S  N  L  S  M  Q  T  H  A  A  R  I  R  T  F  S  N  W  -

    CCTTCTAGTGCACTAGTTCATTCCCAGGAACTTGCAAGTGCGGGCTTTTATTATACAGGA
961 ---------+---------+---------+---------+---------+---------+ 1020
    P  S  S  A  L  V  H  S  Q  E  L  A  S  A  G  F  Y  Y  T  G  -

    CACAGTGATGATGTCAAGTGTTTATGCTGTGATGGTGGGCTGAGGTGCTGGGAATCTGGA
1021 ---------+---------+---------+---------+---------+---------+ 1080
    H  S  D  D  V  K  C  L  C  C  D  G  G  L  R  C  W  E  S  G  -

    GATGACCCCTGGGTGGAACATGCCAAGTGGTTTCCAAGGTGTGAGTACTTGCTCAGAATC
1081 ---------+---------+---------+---------+---------+---------+ 1140
    D  D  P  W  V  E  H  A  K  W  F  P  R  C  E  Y  L  L  R  I  -

    AAAGGCCAAGAATTTGTCAGCCAAGTTCAAGCTGGCTATCCTCATCTACTTGAGCAGCTA
1141 ---------+---------+---------+---------+---------+---------+ 1200
    K  G  Q  E  F  V  S  Q  V  Q  A  G  Y  P  H  L  L  E  Q  L  -
```

FIG. 5 (PAGE 3 OF 6)

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M-hiap-1

```
     TTATCTACGTCAGACTCCCCAGAAGATGAGAATGCAGACGCAGCAATCGTGCATTTTGGC
1201 ---------+---------+---------+---------+---------+---------+ 1260
     L  S  T  S  D  S  P  E  D  E  N  A  D  A  A  I  V  H  F  G   -

     CCTGGAGAAAGTTCGGAAGATGTCGTCATGATGAGCACGCCTGTGGTTAAAGCAGCCTTG
1261 ---------+---------+---------+---------+---------+---------+ 1320
     P  G  E  S  S  E  D  V  V  M  M  S  T  P  V  V  K  A  A  L   -

     GAAATGGGCTTCAGTAGGAGCCTGGTGAGACAGACGGTTCAGTGGCAGATCCTGGCCACT
1321 ---------+---------+---------+---------+---------+---------+ 1380
     E  M  G  F  S  R  S  L  V  R  Q  T  V  Q  W  Q  I  L  A  T   -

     GGTGAGAACTACAGGACCGTCAGTGACCTCGTTATAGGCTTACTCGATGCAGAAGACGAG
1381 ---------+---------+---------+---------+---------+---------+ 1440
     G  E  N  Y  R  T  V  S  D  L  V  I  G  L  L  D  A  E  D  E   -

     ATGAGAGAGGAGCAGATGGAGCAGGCGGCCGAGGAGGAGGAGTCAGATGATCTAGCACTA
1441 ---------+---------+---------+---------+---------+---------+ 1500
     M  R  E  E  Q  M  E  Q  A  A  E  E  E  E  S  D  D  L  A  L   -

     ATCCGGAAGAACAAAATGGTGCTTTTCCAACATTTGACGTGTGTGACACCAATGCTGTAT
1501 ---------+---------+---------+---------+---------+---------+ 1560
     I  R  K  N  K  M  V  L  F  Q  H  L  T  C  V  T  P  M  L  Y   -
```

FIG. 5 (PAGE 4 OF 6)

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M-hiap-1

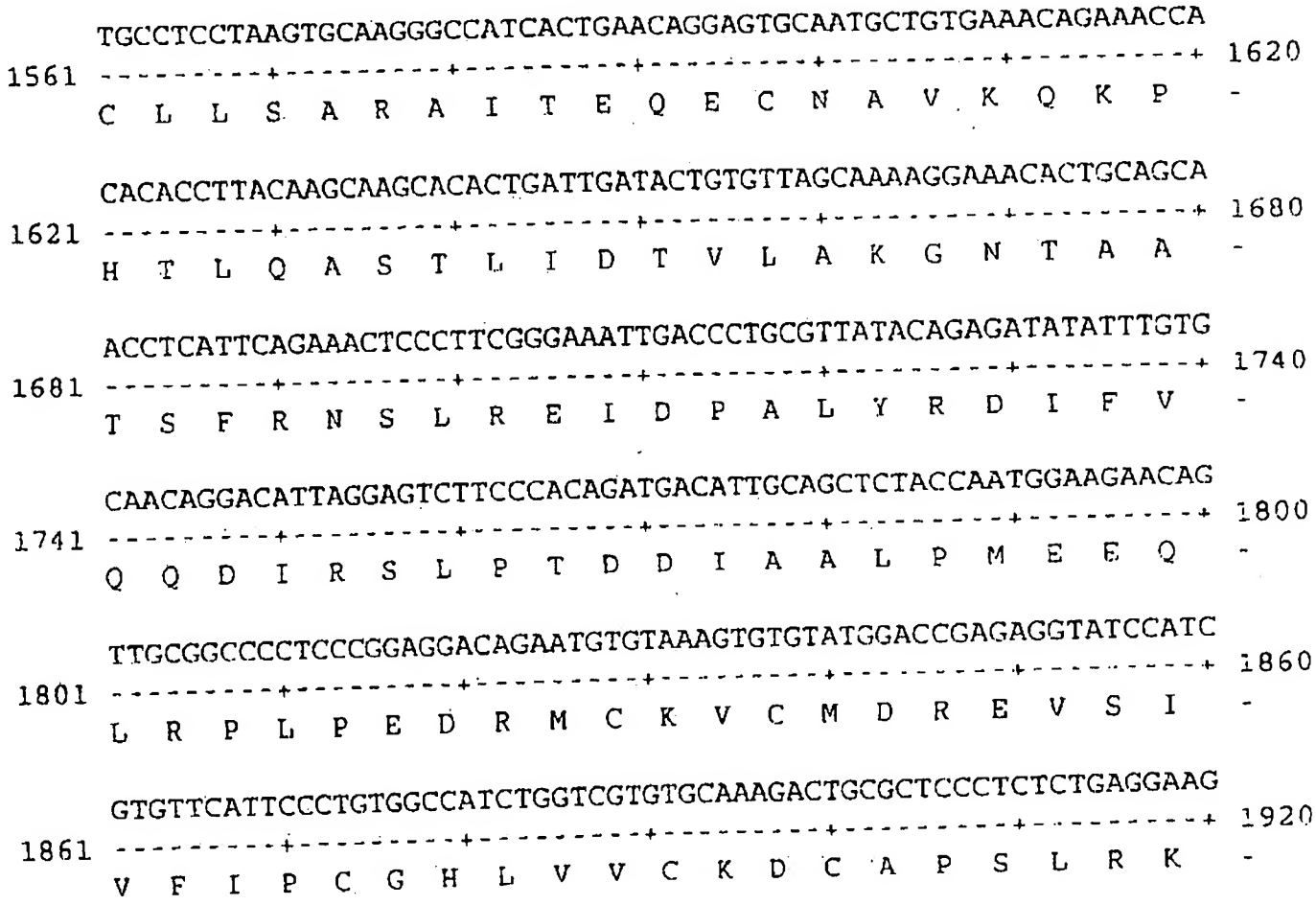

FIG. 5 (PAGE 5 OF 6)

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14.01.97

M-hiap-1

```
     TGTCCCATCTGTAGAGGGACCATCAAGGGCACAGTGCGCACATTTCTCTCCTGAACAAGA
1921 ---------+---------+---------+---------+---------+-------- -+ 1980
     C  P  I  C  R  G  T  I  K  G  T  V  R  T  F  L  S  *         -

     CTAATGGTCCATGGCTGCAACTTCAGCCAGGAGGAAGTTCACTGTCACTCCCAGTTCCAT
1981 ---------+---------+---------+---------+---------+---------+ 2040
     TCGGAACTTGAGGCCAGCCTGGATAGCACGAGACACCGCCAAACACACAAATATAAACAT
2041 ---------+---------+---------+---------+---------+---------+ 2100
     GAAAAACTTTTGTCTGAAGTCAAGAATGAATGAATTACTTATATAATAATTTTAATTGGT
2101 ---------+---------+---------+---------+---------+---------+ 2160
     TTCCTTAAAAGTGCTATTTGTTCCCAACTCAGAAAATTGTTTTCTGTAAACATATTTACA
2161 ---------+---------+---------+---------+---------+---------+ 2220
     TACTACCTGCATCTAAAGTATTCATATATTCATATATTCAGATGTCATGAGAGAGGGTTT
2221 ---------+---------+---------+---------+---------+---------+ 2280
     TGTTCTTGTTCCTGAAAAGCTGGTTTATCATCTGATCAGCATATACTGCGCAACGGGCAG
2281 ---------+---------+---------+---------+---------+---------+ 2340
     GGCTAGAATCCATGAACCAAGCTGCAAAGATCTCACGCTAAATAAGGCGGAAAGATTTGG
2341 ---------+---------+---------+---------+---------+---------+ 2400
     AGAAACGAAAGGAAATTCTTTCCTGTCCAATGTATACTCTTCAGACTAATGACCTCTTCC
2401 ---------+---------+---------+---------+---------+---------+ 2460
     TATCAAGCCTTCTA
2461 ---------+---- 2474
```

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M-hiap-2

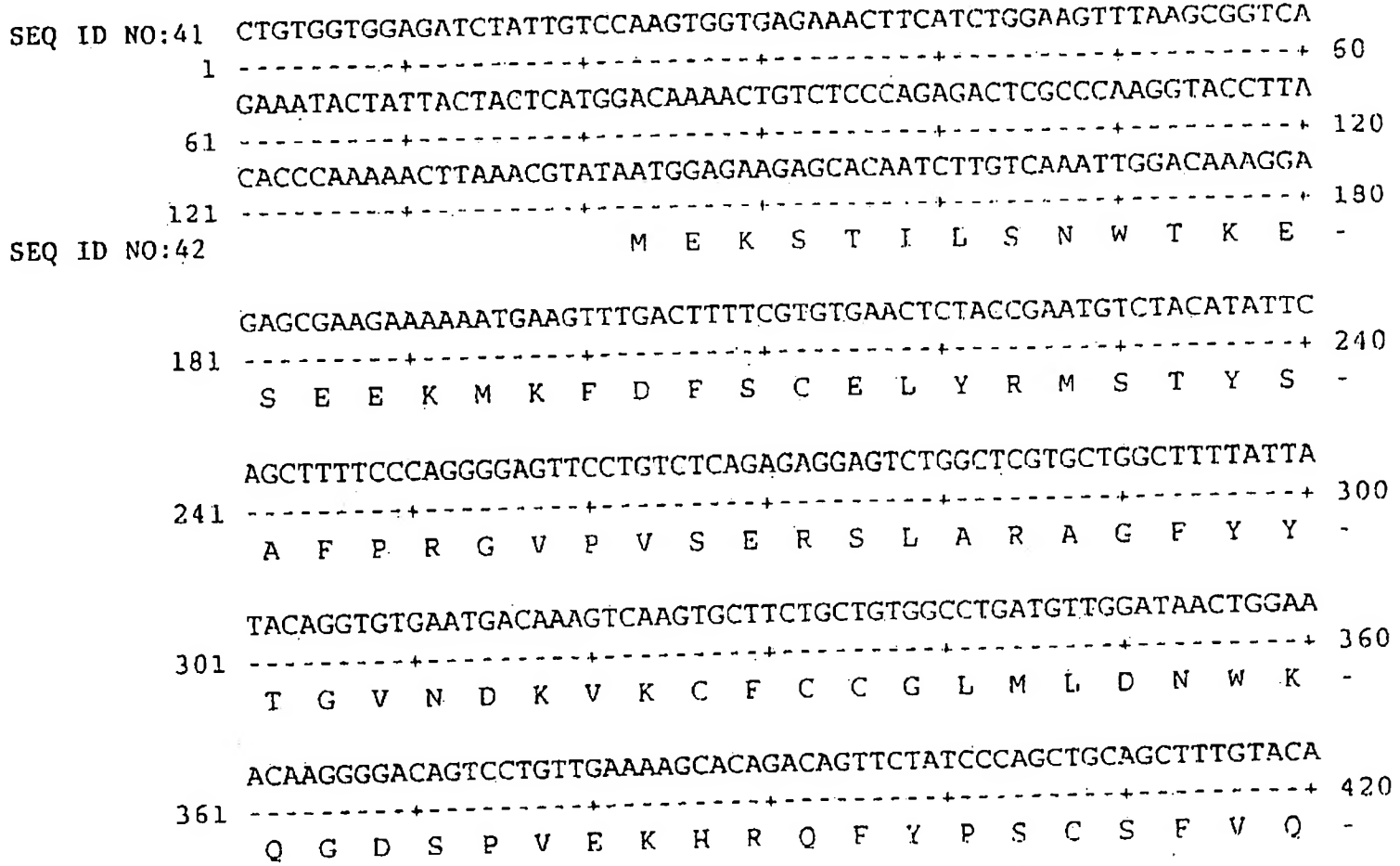

FIG. 6 (PAGE 1 OF 6)

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14.01.97

M-hiap-2

```
    GACTCTGCTTTCAGCCAGTCTGCAGTCTCCATCTAAGAATATGTCTCCTGTGAAAAGTAG
421 ---------+---------+---------+---------+---------+---------+ 480
     T  L  L  S  A  S  L  Q  S  P  S  K  N  M  S  P  V  K  S  R  -

    ATTTGCACATTCGTCACCTCTGGAACGAGGTGGCATTCACTCCAACCTGTGCTCTAGCCC
481 ---------+---------+---------+---------+---------+---------+ 540
     F  A  H  S  S  P  L  E  R  G  G  I  H  S  N  L  C  S  S  P  -

    TCTTAATTCTAGAGCAGTGGAAGACTTCTCATCAAGGATGGATCCCTGCAGCTATGCCAT
541 ---------+---------+---------+---------+---------+---------+ 600
     L  N  S  R  A  V  E  D  F  S  S  R  M  D  P  C  S  Y  A  M  -

    GAGTACAGAAGAGGCCAGATTTCTTACTTACAGTATGTGGCCTTTAAGTTTTCTGTCACC
601 ---------+---------+---------+---------+---------+---------+ 660
     S  T  E  E  A  R  F  L  T  Y  S  M  W  P  L  S  F  L  S  P  -

    AGCAGAGCTGGCCAGAGCTGGCTTCTATTACATAGGGCCTGGAGACAGGGTGGCCTGTTT
661 ---------+---------+---------+---------+---------+---------+ 720
     A  E  L  A  R  A  G  F  Y  Y  I  G  P  G  D  R  V  A  C  F  -

    TGCCTGTGGTGGGAAACTGAGCAACTGGGAACCAAAGGATTATGCTATGTCAGAGCACCG
721 ---------+---------+---------+---------+---------+---------+ 780
     A  C  G  G  K  L  S  N  W  E  P  K  D  Y  A  M  S  E  H  R  -
```

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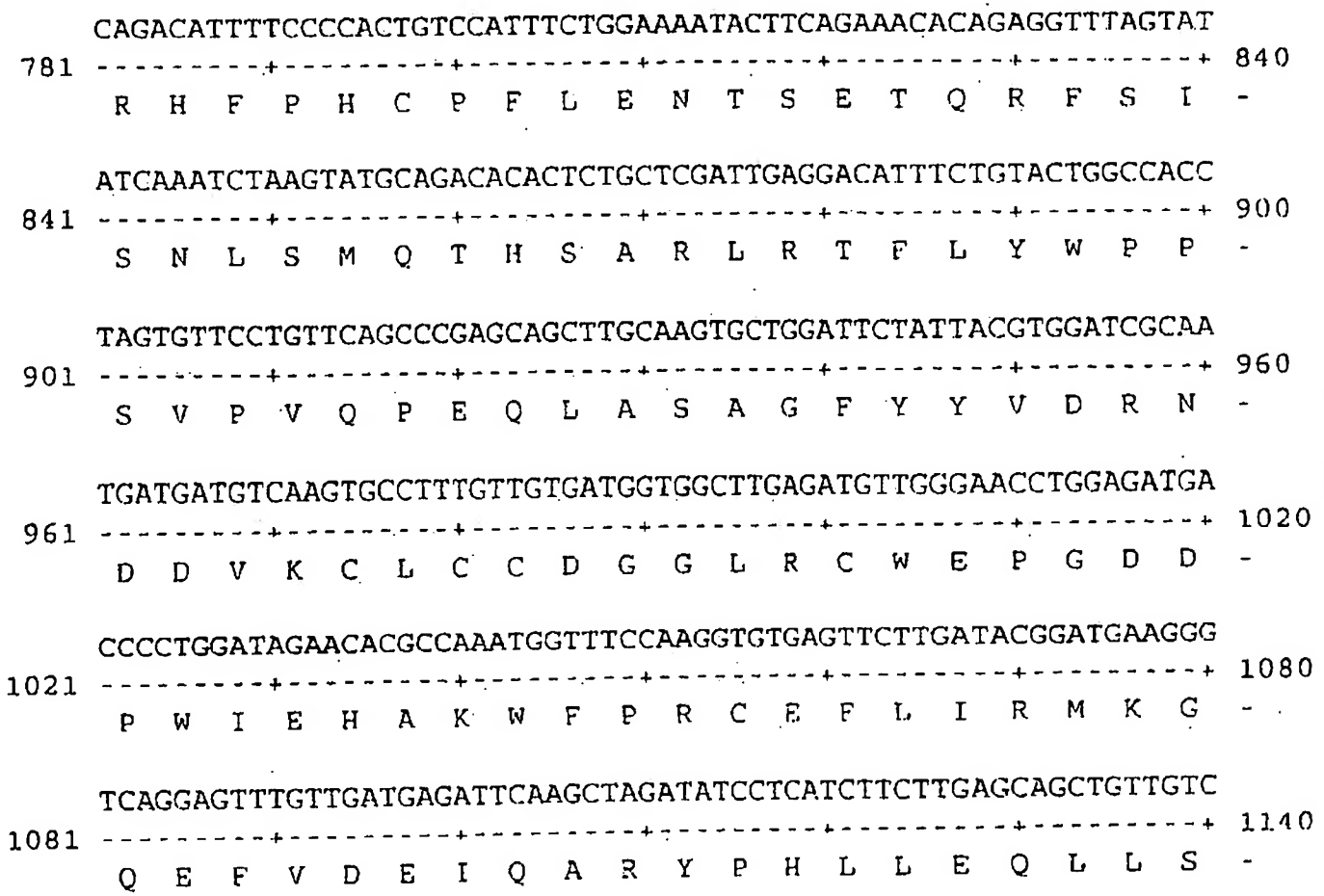
M-hiap-2



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14.01.97

M-hiap-2

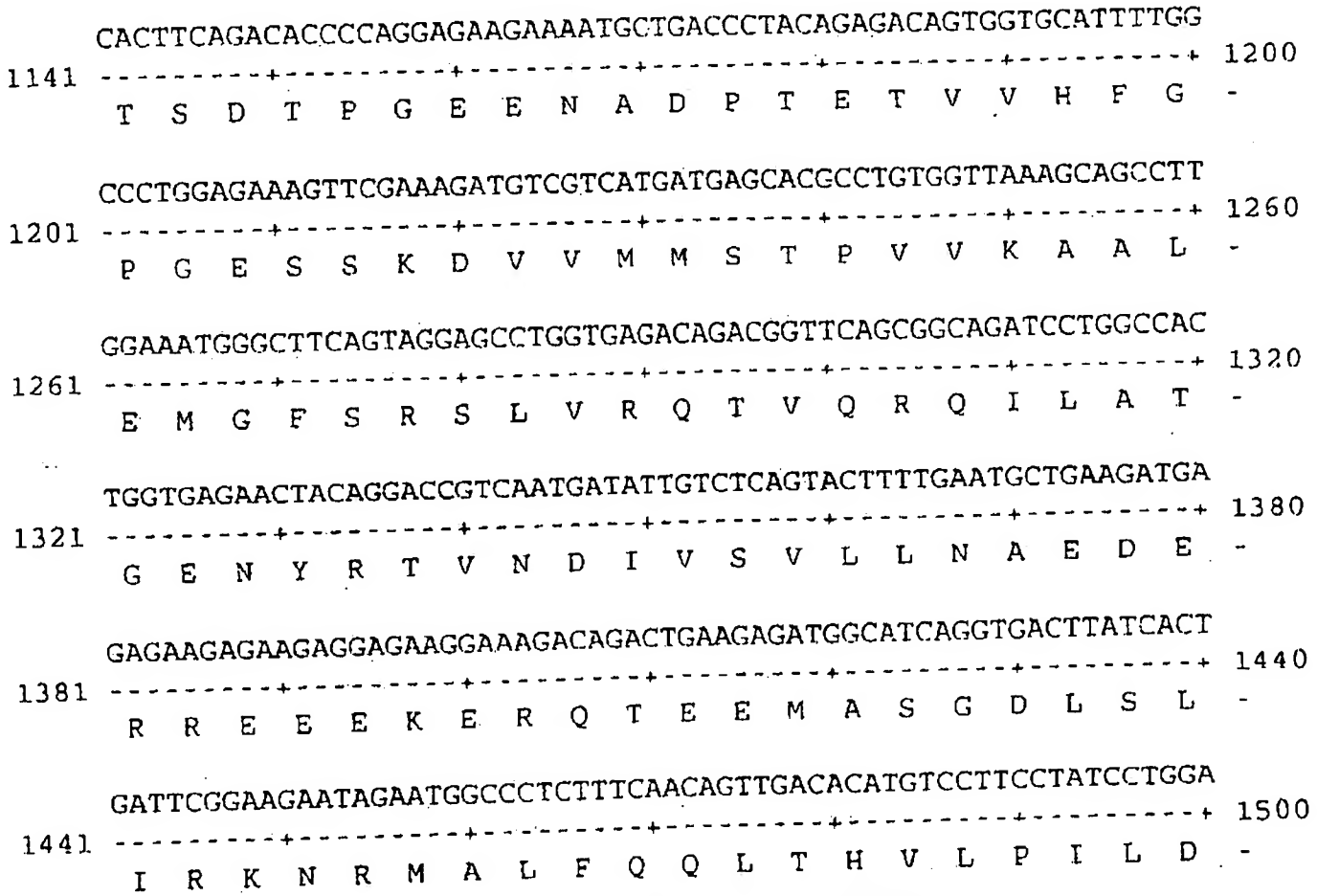

FIG. 6 (PAGE 4 OF 6)

M-hiap-2

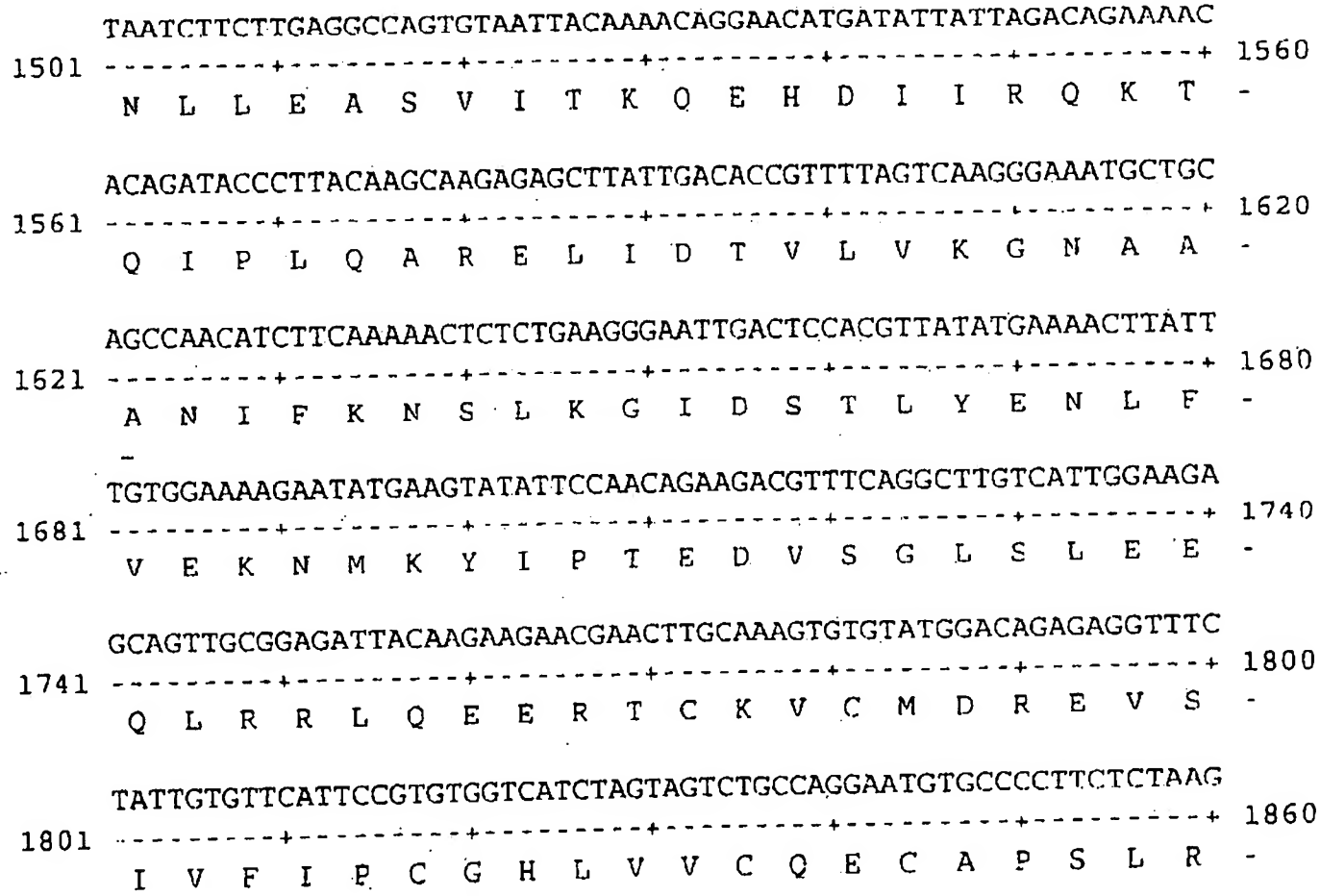

FIG. 6 (PAGE 5 OF 6)

M-hiap-2

```
     GAAGTGCCCCATCTGCAGGGGGACAATCAAGGGGACTGTGCGCACATTTCTCTCATGAGT
1861 ---------+---------+---------+---------+---------+---------+ 1920
      K  C  P  I  C  R  G  T  I  K  G  T  V  R  T  F  L  S  *     -

     GAAGAATGGTCTGAAAGTATTGTTGGACATCAGAAGCTGTCAGAACAAAGAATGAACTAC
1921 ---------+---------+---------+---------+---------+---------+ 1980
     TGATTTCAGCTCTTCAGCAGGACATTCTACTCTCTTTCAAGATTAGTAATCTTGCTTTAT
1981 ---------+---------+---------+---------+---------+---------+ 2040
     GAAGGGTAGCATTGTATATTTAAGCTTAGTCTGTTGCAAGGGAAGGTCTATGCTGTTGAG
2041 ---------+---------+---------+---------+---------+---------+ 2100
     CTACAGGACTGTGTCTGTTCCAGAGCAGGAGTTGGGATGCTTGCTGTATGTCCTTCAGGA
2101 ---------+---------+---------+---------+---------+---------+ 2160
     CTTCTTGGGATTTGGGAATTTGGGGAAAGCTTTGGAATCCAGTGATGTGGAGCTCAGAAA
2161 ---------+---------+---------+---------+---------+---------+ 2220
     TCCTGGAACCAGTGACTCTGGTACTCAGTAGATAGGGTACCCTGTACTTCTTGGTGCTTT
2221 ---------+---------+---------+---------+---------+---------+ 2280
     TCCAGTCTGGGAAATAAGGAGGAATCTGCTGCTGGTAAAAATTTGCTGGATGTGAGAAAT
2281 ---------+---------+---------+---------+---------+---------+ 2340
     AGATGAAAGTGTTTCGGGTGGGGGCGTGCATCAGTGTAGTGTGTGCAGGGATGTATGCAG
2341 ---------+---------+---------+---------+---------+---------+ 2400
     GCCAAACACTGTGTAG
2401 ---------+------ 2416
```

FIG. 6 (PAGE 6 OF 6)

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Alignment of BIR (Baculoviral IAP Repeats) Domains

Baculovirus	
Cp_iap	Cydia pomonella
Op_iap	Orgyia pseudotsugata
Human	
xiap	IAP on X chromosome
hiap1, hiap2	two different human IAP genes
Mouse	
m-xiap	mouse homologue of human xiap gene
Insect	
diap	Drosophilia IAP gene, not clearly a homologue of xiap or hiap

FIG. 7

note on consensus: The consensus line represents amino acids or very similar amino acids which are present in 14 of the 19 BIR sequences at each position. Capitalized residues are those that are in the consensus sequence.

```
                              1                                                                            68
SEQ ID NO:11   Op_iap-1       ksaRLgTYtn WPvqf.lsps rMAseGFYYl GrgDeVrCaf CkveitnWvr gDdpeLdHkr waPqCpFV
SEQ ID NO:14   Cp_iap-1       esvRLnTFek WPvsf.lsps tMAknGFYYl GrsDeVrCaf CkveimrWke gEdpsadHkk waPqCpFV
SEQ ID NO:15   diap-2         esnRLvTFkd WPnpn.iLpq aLAkAGFYYl nrlDhVkCvw Cngviakwek nDnafeeHkr ffPqCprV
SEQ ID NO:16   m-xiap-1       efnRLkTFan FPsespvsas tLArAGFLYt GegDtVqCFs ChaaidrWqy gDsavgrHrr isPnCrFI
SEQ ID NO:17   xiap-1         efnRLkTFan FPsgspvsas tLArAGFLYt GegDtVrCFs ChaavdrWqy gDsavgrHrk vsPnCrFI
SEQ ID NO:18   hiap1-1        elyRMsTYst FPagvpvser sLArAGFYYt GvnDkVkCFc CglmldnWkr gDsptekHkk lyPsCrFV
SEQ ID NO:19   hiap2-1        elyRMsTYst FPagvpvser sLArAGFYYt GvnDkVkCFc CglmldnWkl gDspiqkHkq lyPsCsFI
SEQ ID NO:20   m-xiap-2       essRLksFqn WPdyahltpr eLAsAGLYYt GadDqVqCFc CggklknWep cDrawseHrr hfPnCfFV
SEQ ID NO:21   xiap-2         essRLksFqn WPdyahltpr eLAsAGLYYt GigDqVqCFc CggklknWep cDrawseHrr hfPnCfFV
SEQ ID NO:22   hiap1-2        enaRLlTFqt WP.lLflspt dLArAGFYYi GpgDrVaCFa CggklsnWep kDnamseHlr hfPkCpFI
SEQ ID NO:23   hiap2-2        esaRFlTYhm WP.lLflsps eLArAGFYYi GpgDrVaCFa CggklsnWep kDdamseHrr hfPnCpFl
SEQ ID NO:24   m-xiap-3       ysaRivTFgt Wiysv..nke qLArAGFYal GegDkVkCFh CgggltdWkp sEdpwdqHak cyPgCkYl
SEQ ID NO:25   xiap-3         yeaRifTFgt Wiysv..nke qLArAGFYal GegDkVkCFh CgggltdWkp sEdpweqHak wyPgCkYl
SEQ ID NO:26   hiap1-3        hasRFkTFfn WPssvlvnps qLAsAGFYYv GnsDdVkCFc CdgglrcWes gDdpwvqHak wfPrCeYl
SEQ ID NO:27   hiap2-3        hasRMrTFmy WPssvpvqpe qLAsAGFYYv GrnDdVkCFg CdgglrcWes gDdpwveHak wfPrCeFl
SEQ ID NO:28   Op_iap-2       esaRLrTFae WPrglkqrps eLAsAGFFYt GqgDktrCFc CdgglkdWep dDapwqqHar wydrCeYV
SEQ ID NO:29   Cp_iap-2       esaRvksFhn WPrcmkqrps qMAdAGFFYt GygDntkCFy CdgglkdWep eDvpweqHvr widrCaYV
SEQ ID NO:30   diap-3         vdaRLrTFtd WPisniqpas aLAqAGLYYq kigDqVrCFh CniglrsWqk eDepwieHak wsPkCqFV
SEQ ID NO:31   diap-1         esvRLaTFge WPlnapvsae dLvanGFF.. GtwmeaeCdf ChvridrWey gDlvaerHrr ssPiCsmV
               Consensus      ---RL-TF-- WP-------- -LA-AGFYY- G--D-V-CF- C------W-- -D-----H-- --P-C-FV
```


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14.01.97

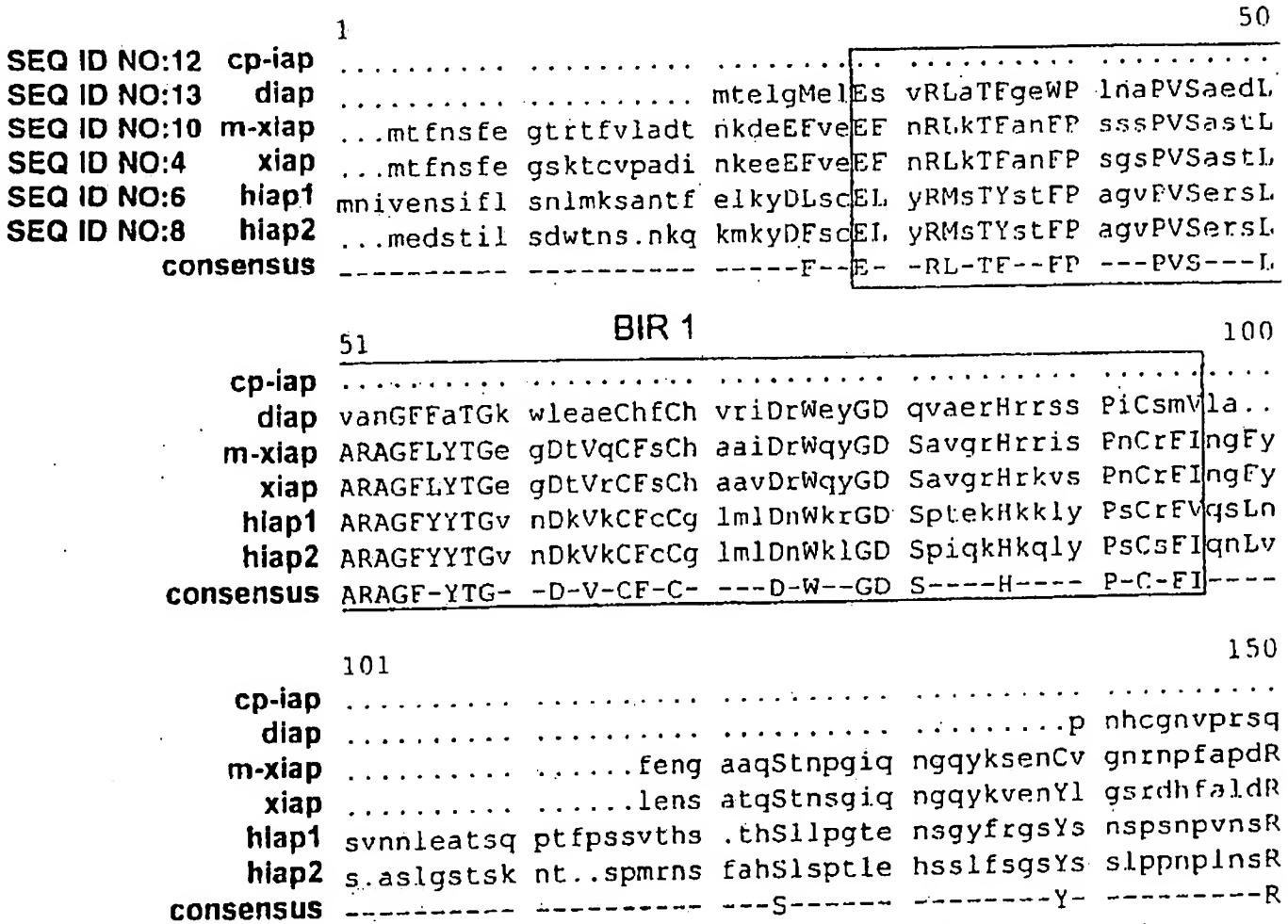

FIG. 8 (PAGE 1 OF 5)

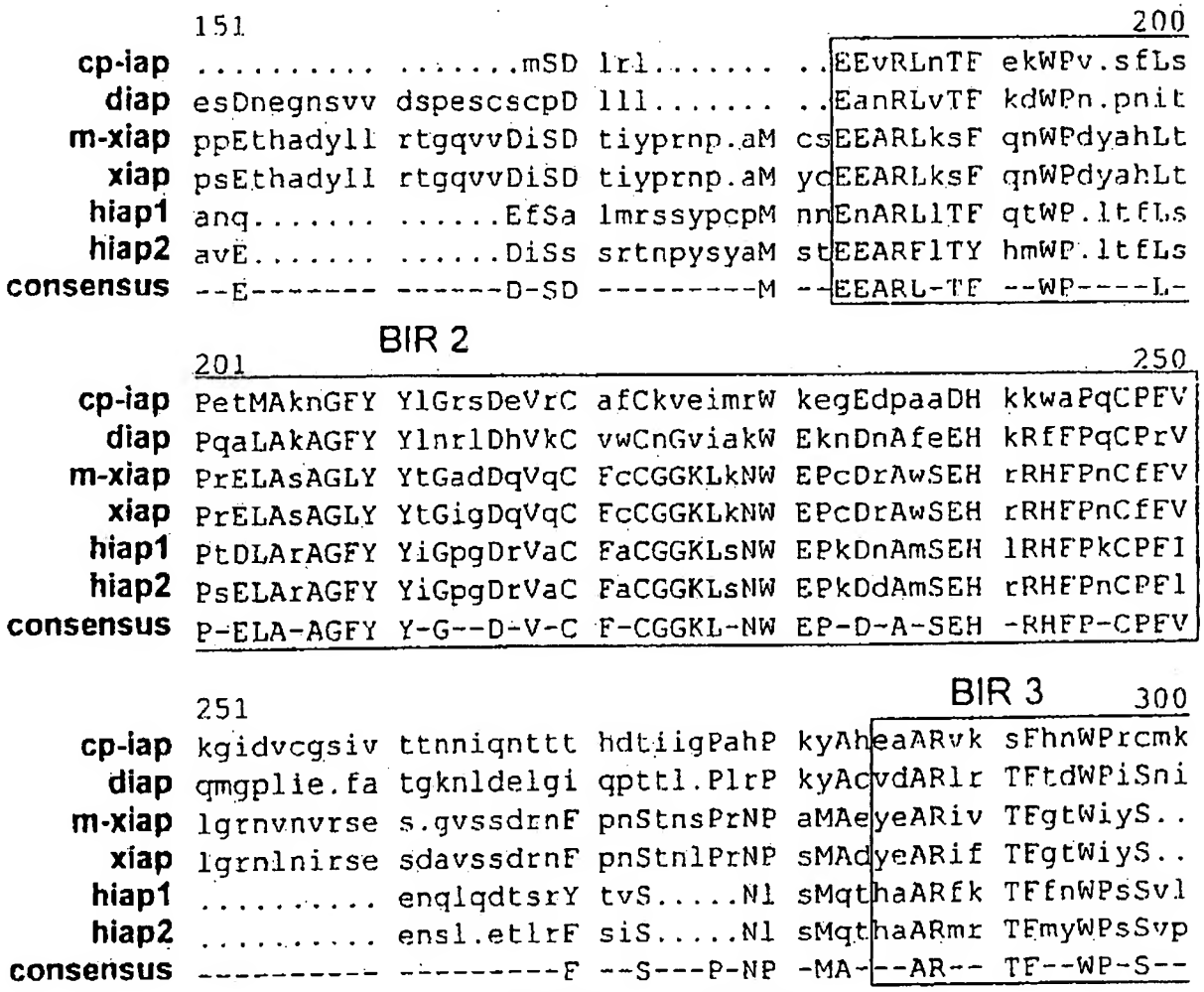

FIG. 8 (PAGE 2 OF 5)

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PCT/IB 96/01022
14.01.97

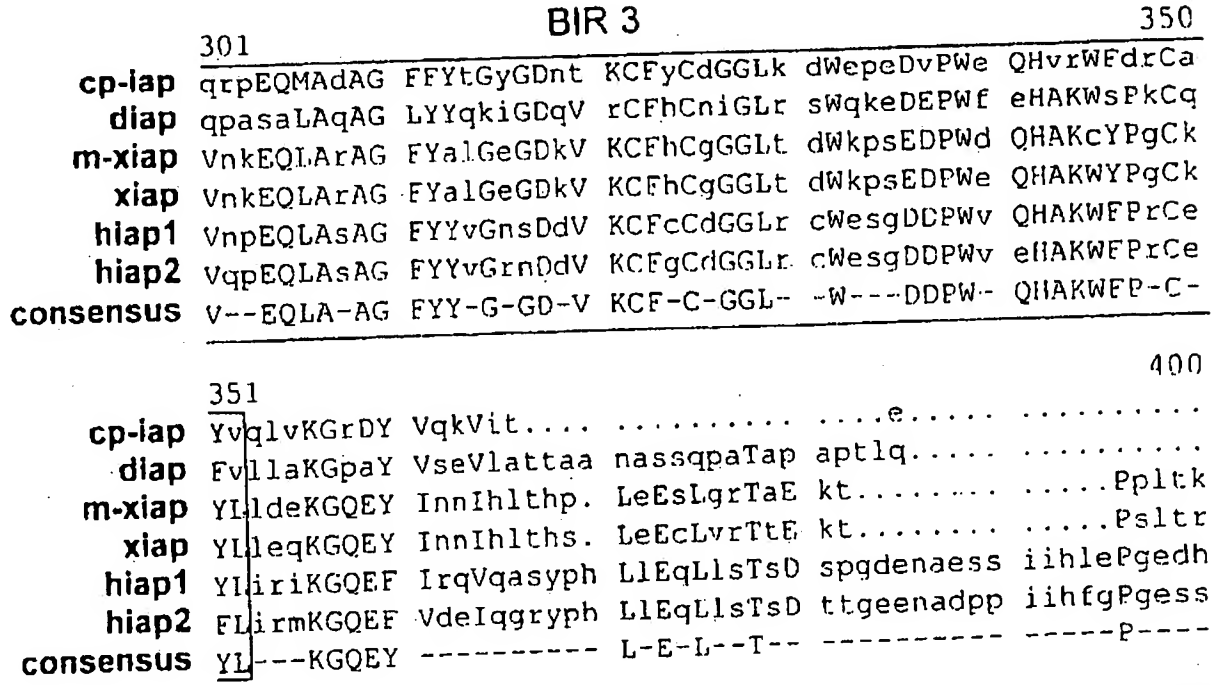

FIG. 8 (PAGE 3 OF 5)

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14.01.97


```
                451                                                        500
cp-iap      .......... .......... .......... .......... ..........
diap        fddagagaal Evreppe... .......... .......... ..........
m-xiap      vsAqkDnteD E......... .......... .......... ..........
xiap        vnAqkDsmqD E......... .......... .......... ..........
hiap1       lnAedEireE Ererateeke sndllllirkn rmalfqhltc vipildslLt
hiap2       lnAedEkreE Ekekqaeema sddlslirkn rmalfqqltc vlpildnllk
consensus   --A------- E--------- ---------- ---------- ----------

                501                                                        550
cp-iap      .......... .......... ..nttvstaa pvsepipe.. ..........
diap        .......... ...psapfie pcqattskaa svpipvadsi pakpqaaeav
m-xiap      .......... ....ssQtsL Q......... .......... ..........
xiap        .......... ....ssQtsL Q......... .......... ..........
hiap1       agiineqehd vikqktQtsL Qarelidtil vkgniaatvf rnslqeaeav
hiap2       anvinkqehd iikqktQipL Qarelidtiw vkgnaaanif knclkeidst
consensus   ---------- ------Q--L Q--------- ---------- ----------
```

FIG. 8 (PAGE 4 OF 5)

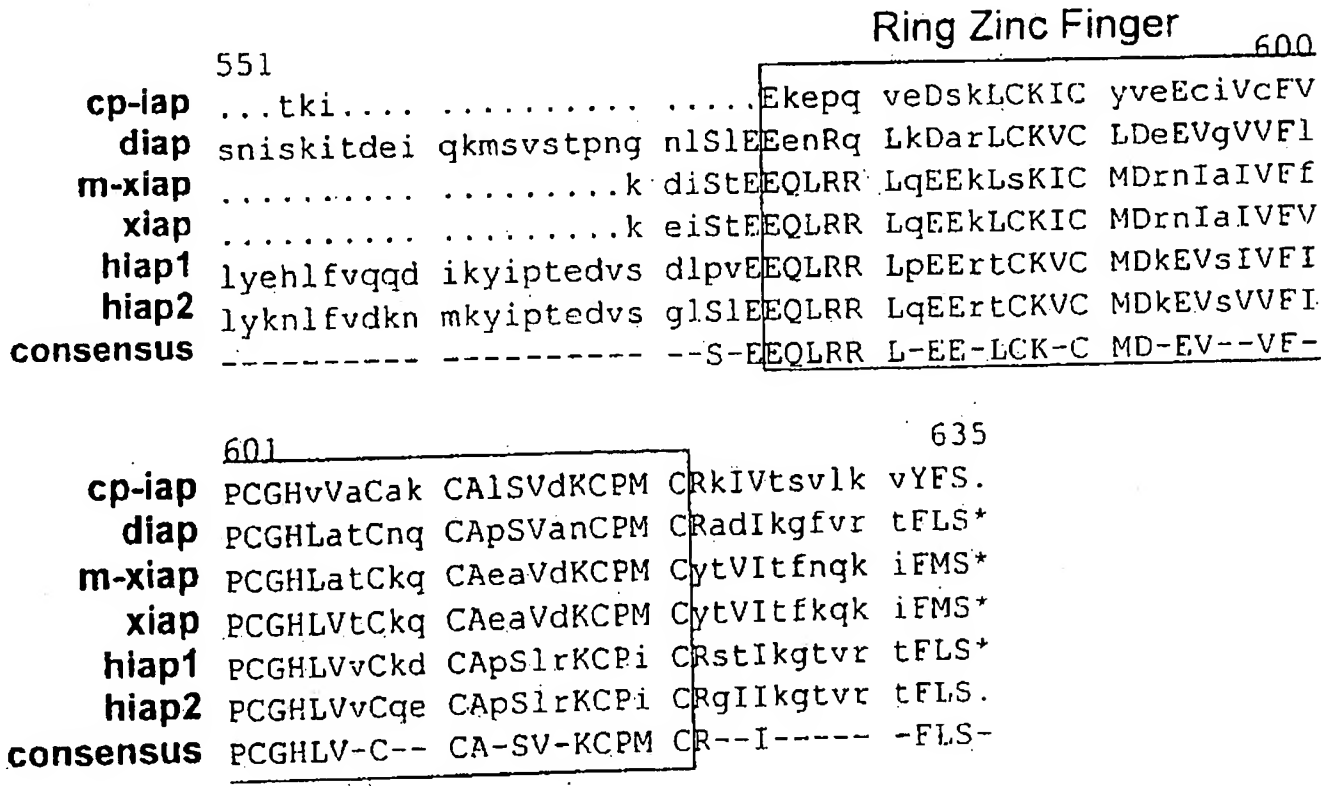

FIG. 8 (PAGE 5 OF 5)

PCT/IB 96/01022
14. 01. 97

Alignment of RZF (Ring Zinc Finger) Domains

Baculovirus
- Cp_iap — Cydia pomonella
- Op_ap — Orgyia pseudotsugata

Human
- xiap — IAP on X chromosome
- hiap1, hiap2 — two different human IAP genes

Mouse
- m-xiap — mouse homologue of human xiap gene

Insect
- diap — Drosophilia IAP gene, not clearly a homologue of xiap or hiap

note on consensus: The consensus line represents amino acids or very similar amino acids which are present in 6 of the 7 RZF sequences at each position. Capitalized residues are those that are in the consensus sequence.

```
                                 1                                                    46
SEQ ID NO:32  hiap2              EqlrrlqEer tCKVCMdkev sVvFiPCGHl vvCqeCApel rkCPiC
SEQ ID NO:33  hiap1              EqltrlpEer tCKVCMdkev sIvFiPCGHl w CkdCApsl rkCPiC
SEQ ID NO:34  m-xiap             EqltrlqEek lsKICMdrni aIvFfPCGHl atCkqCAeav dkCPmC
SEQ ID NO:35  xiap               EqltrlqEek lCKICMdrni aIvFvPCGHl vtCkqCAeav dkCPmC
SEQ ID NO:36  diap               EenrglkDar lCKVCLdeev gVvFlPCGHl atCnqCApev anCPmC
SEQ ID NO:37  Cp_iap             EkepqveDsk lCKICyveec iVcFvPCGHv vaCakCAlsv dkCPmC
SEQ ID NO:38  Op_iap             aveaevaDdr lCKICLgack tVcFvPCGHv vaCgkCAagv ttCPvC
              Consensus          E------E-- -CKICM---- -V-F-PCGH- --C--CA--- --CP-C
```

FIG. 9

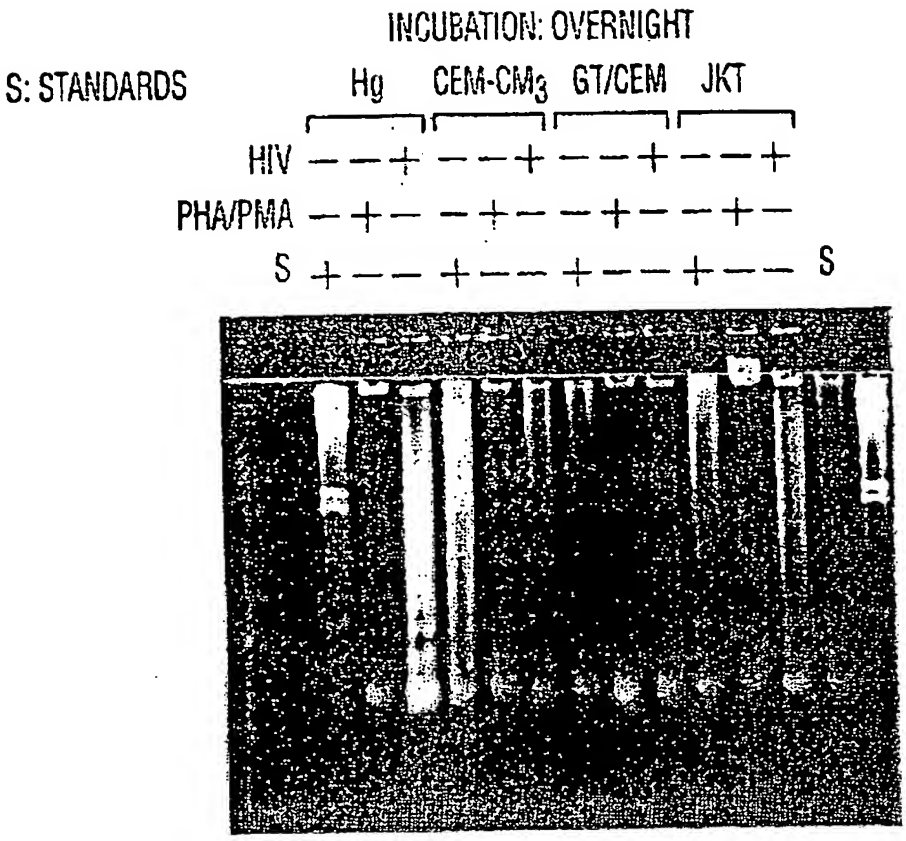

FIG.13A

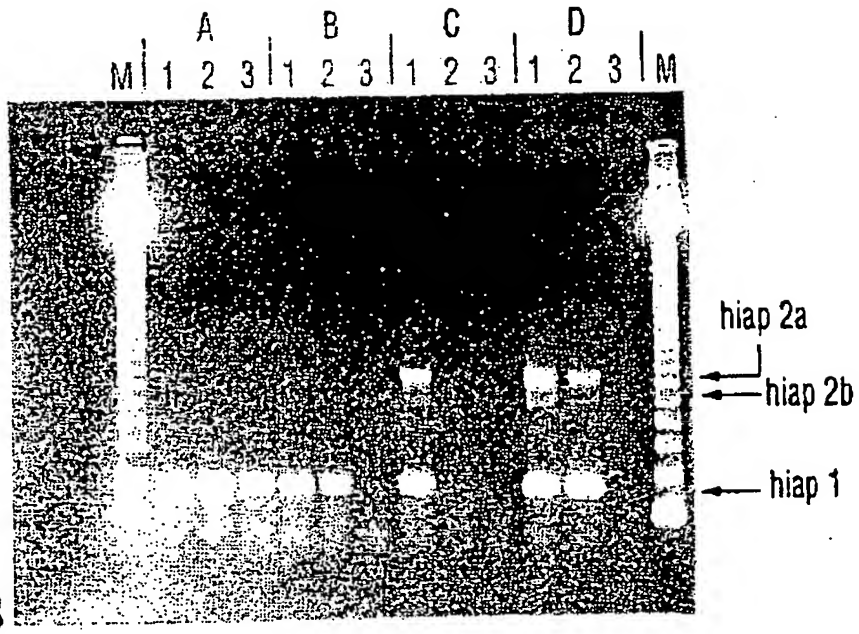

FIG.13B

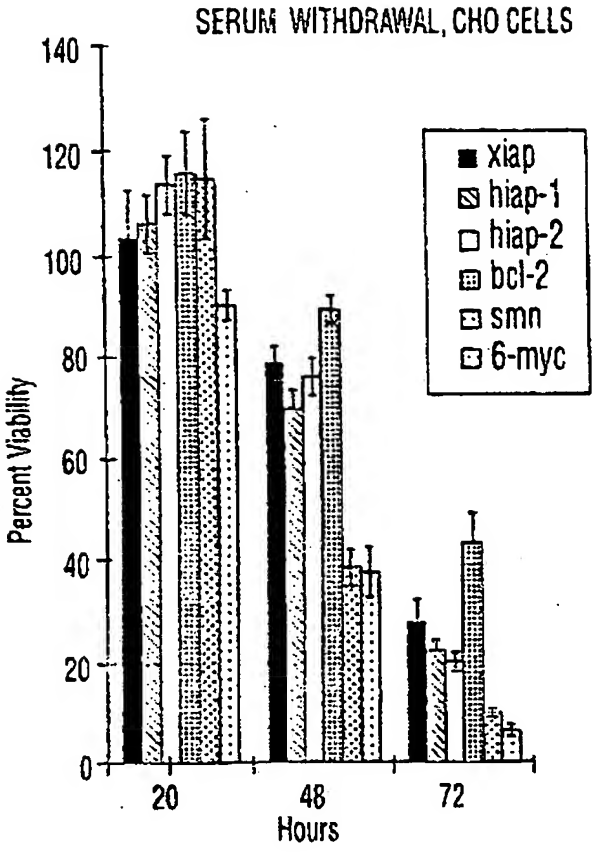

FIG.14A

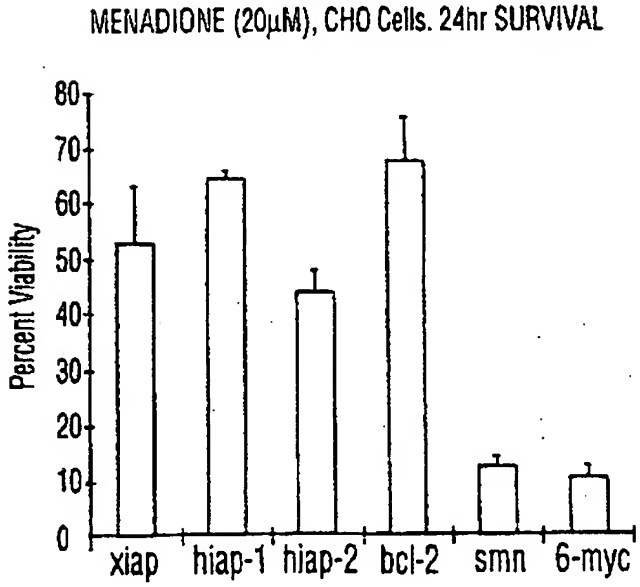

FIG.14B

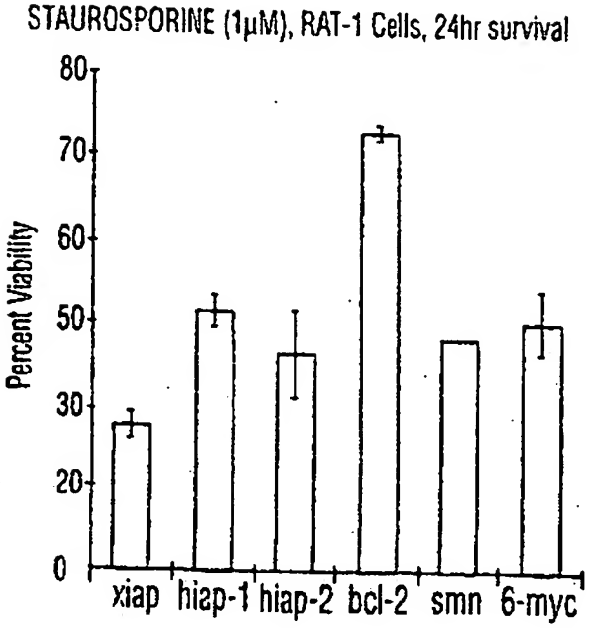
STAUROSPORINE (1μM), RAT-1 Cells, 24hr survival
Percent Viability
80
70
60
50
30
20
0
xiap
hiap-1
hiap-2
bcl-2
smn
6-myc

FIG.14C

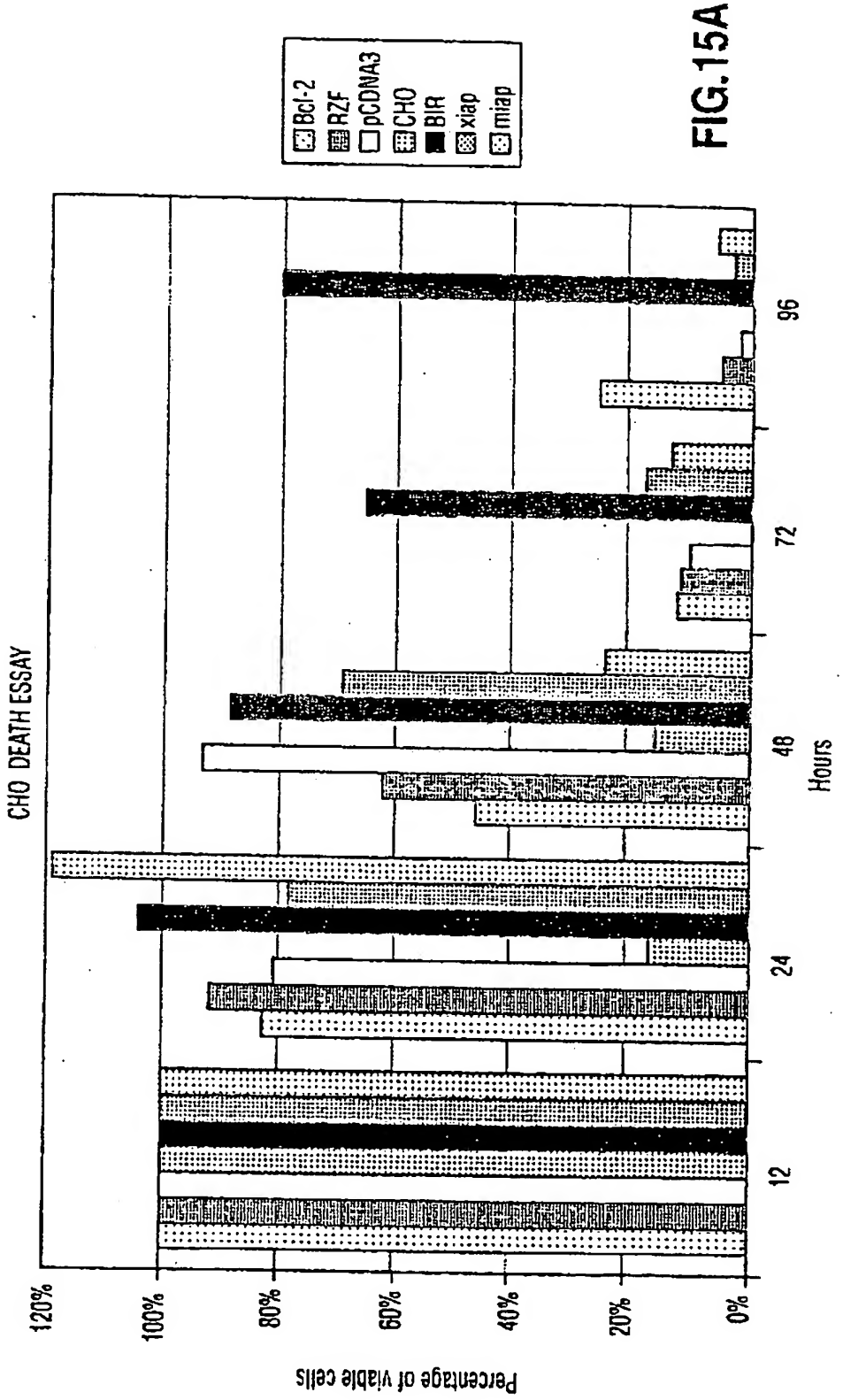
CHO DEATH ESSAY
Bcl-2
RZF
pCDNA3
CHO
BIR
xiap
miap
Percentage of viable cells
120%
100%
80%
60%
40%
20%
0%
12
24
48
72
96
Hours

FIG.15A

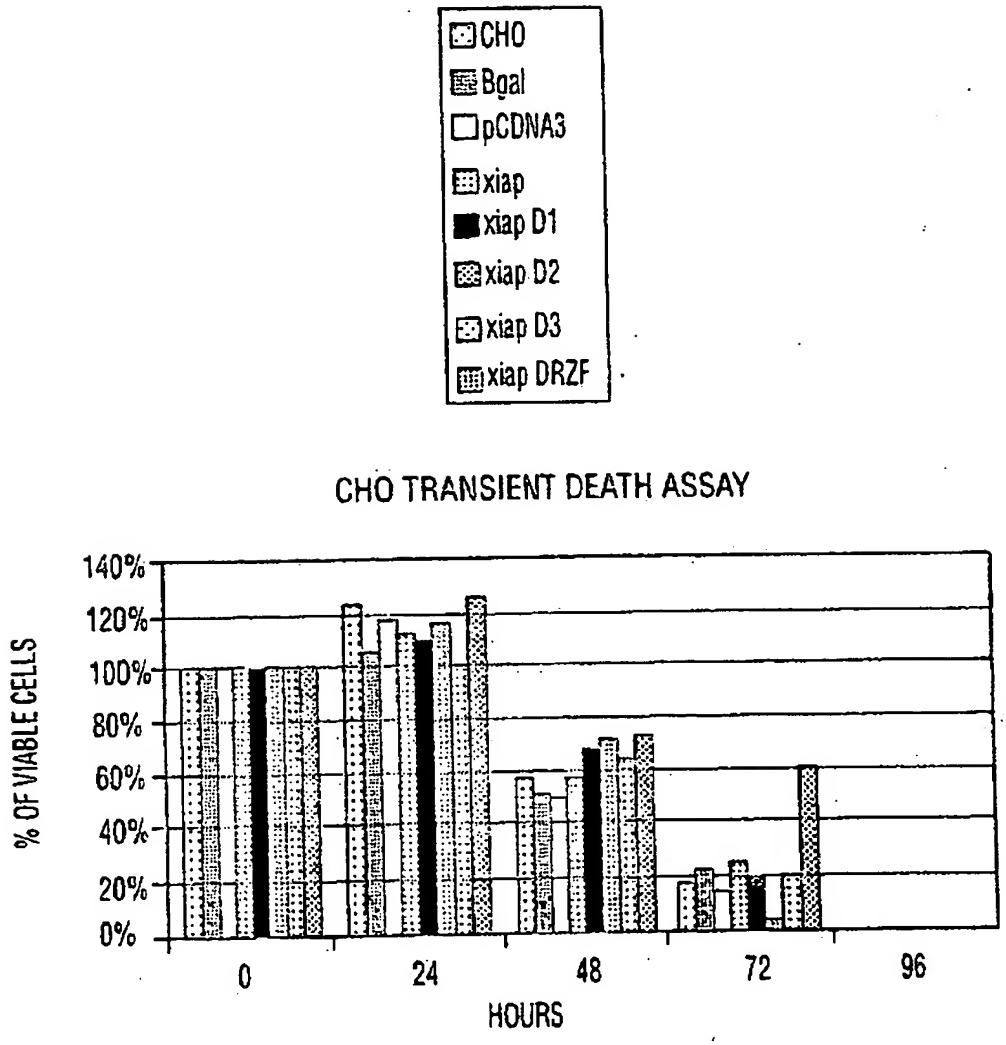
CHO
Bgal
pCDNA3
xiap
xiap D1
xiap D2
xiap D3
xiap DRZF
CHO TRANSIENT DEATH ASSAY
% OF VIABLE CELLS
140%
120%
100%
80%
60%
40%
20%
0%
0
24
48
72
96
HOURS

FIG.15B

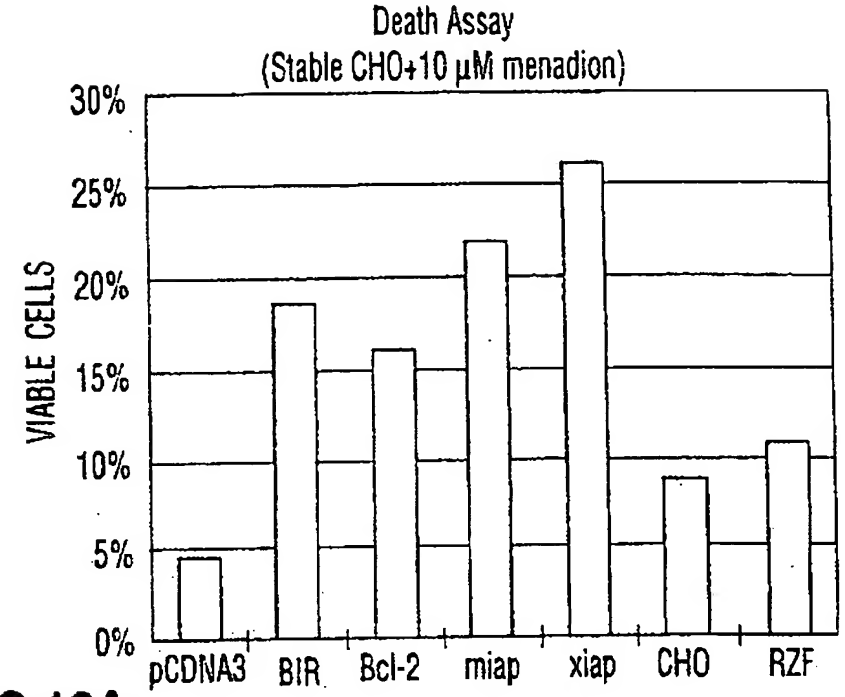

FIG.16A

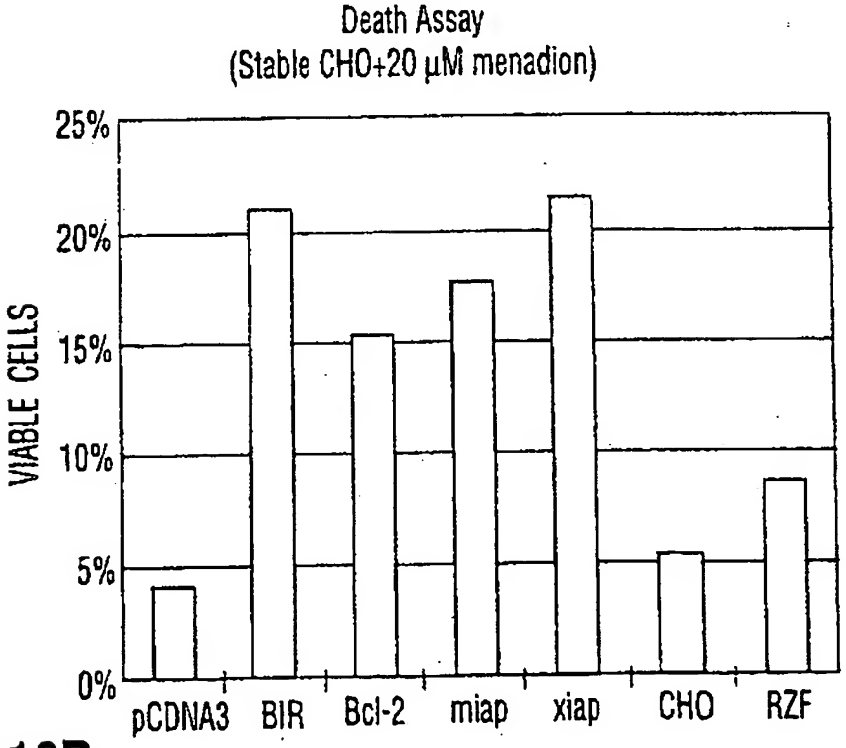

FIG.16B

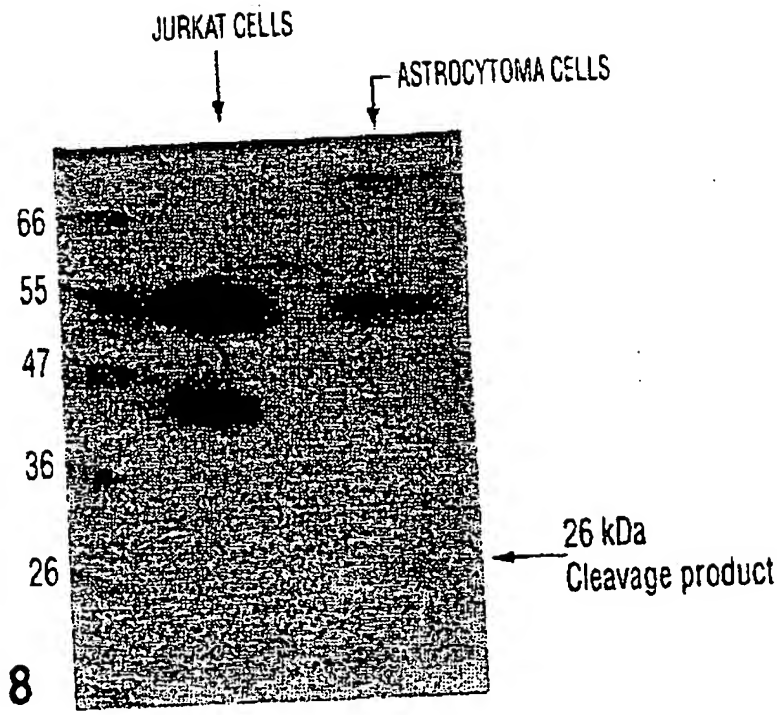
JURKAT CELLS
ASTROCYTOMA CELLS
66
55
47
36
26
26 kDa
Cleavage product

FIG. 18

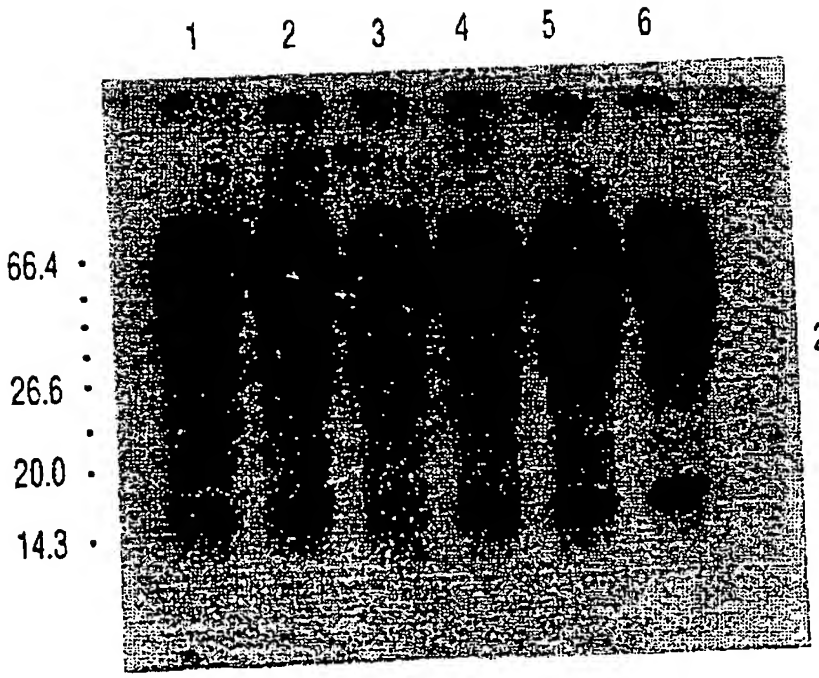

FIG.20

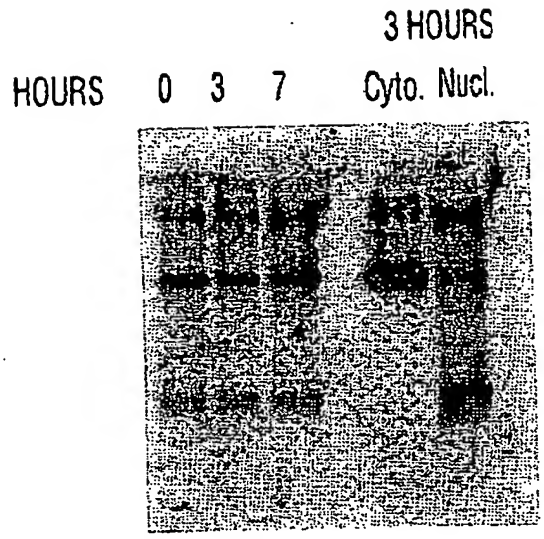

FIG.22A

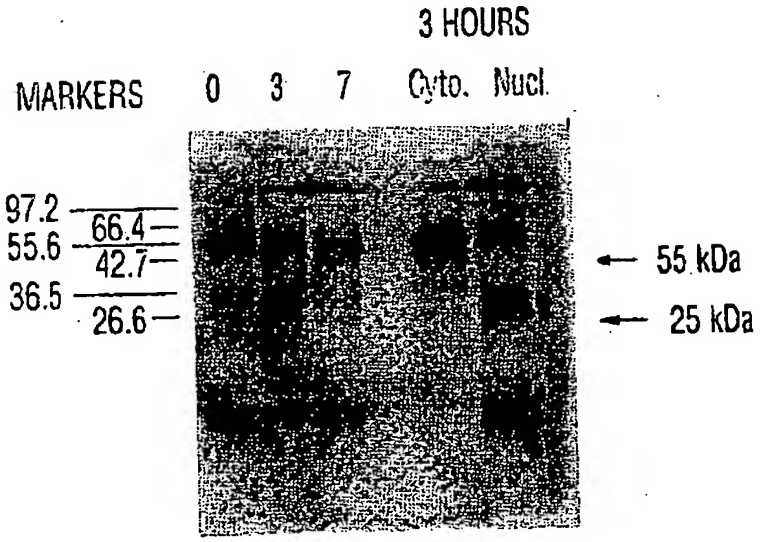

FIG.22B

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/12, C07K 14/47, C12N 5/10, A01K 67/027, A61K 38/17, C12Q 1/68, C07K 16/18, G01N 33/53
A3

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(21) International Application Number: PCT/IB96/01022

(22) International Filing Date: 5 August 1996 (05.08.96)

(30) Priority Data:
08/511,485 4 August 1995 (04.08.95) US
08/576,956 22 December 1995 (22.12.95) US

(60) Parent Applications or Grants
(63) Related by Continuation
US 08/511,485 (CON)
Filed on 4 August 1995 (04.08.95)
US 08/576,956 (CON)
Filed on 22 December 1995 (22.12.95)

(71) Applicant (*for all designated States except US*): UNIVERSITY OF OTTAWA [CA/CA]; 550 Cumberland, Ottawa, Ontario K1N 6N5 (CA).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): KORNELUK, Robert, G. [CA/CA]; 1901 Tweed Avenue, Ottawa, Ontario K1G 2L8 (CA). MACKENZIE, Alexander, E. [CA/CA]; 35 Rockcliffe Way, Ottawa, Ontario K1M 1A3 (CA). BAIRD, Stephen [CA/CA]; 20 Julian Avenue, Ottawa, Ontario K1Y 0S5 (CA). LISTON, Peter [CA/CA]; Children's Hospital of Eastern Ontario, 401 Smyth, Ottawa, Ontario K1H 8L1 (CA).

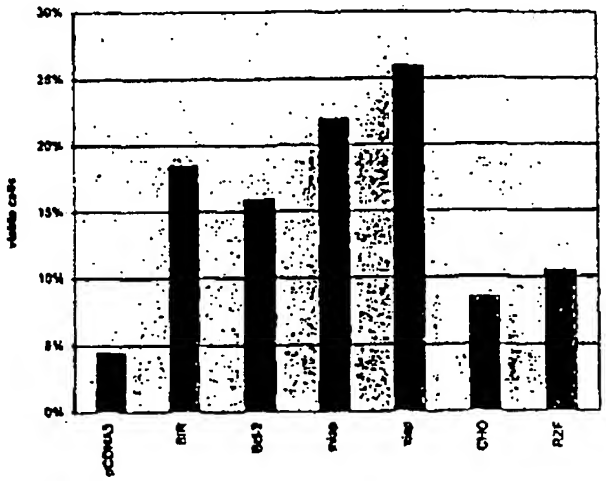
(74) Agent: MORROW, Joy, D.; Smart & Biggar, 900 - 55 Metcalfe Street, P.O. Box 2999, Station D, Ottawa, Ontario K1P 5Y6 (CA).

(81) Designated States: CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.
With a request for rectification under Rule 91.1(f).

(88) Date of publication of the international search report:
30 October 1997 (30.10.97)

(54) Title: MAMMALIAN APOPTOSIS INHIBITOR PROTEIN GENE FAMILY, PRIMERS, PROBES AND DETECTION METHODS



BIR = BACULOVIRUS IAP REPEAT
RZF = RING ZINC FINGER

(57) Abstract

Disclosed is substantially pure DNA encoding mammalian IAP polypeptides; substantially pure polypeptides; and methods of using such DNA to express the IAP polypeptides in cells and animals to inhibit apoptosis. Also disclosed are conserved regions characteristic of the IAP family and primers and probes for the identification and isolation of additional IAP genes. In addition, methods for treating diseases and disorders involving apoptosis are provided.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 96/01022

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/47 C12N5/10 A01K67/027 A61K38/17
C12Q1/68 C07K16/18 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CLEM RJ ET AL: "Control of programmed cell death by the baculovirus genes p35 and iap." MOL CELL BIOL, AUG 1994, 14 (8) P5212-22. UNITED STATES, XP000611843 cited in the application see the whole document	1-3, 17-19, 22,23, 91,92
Y		9-16, 24-38, 49-85, 88,90, 93,94

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 June 1997	17.09.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Gurdjian, D

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INTERNATIONAL SEARCH REPORT

(nternational Application No
PCT/IB 96/01022

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ROY N ET AL: "The gene for neuronal apoptosis inhibitory protein is partially deleted in individuals with spinal muscular atrophy [see comments]" CELL, JAN 13 1995, 80 (1) P167-78, UNITED STATES, XP002032295 see the whole document	9-16, 25-38, 49-85, 88,90, 93,94
Y	WO 95 19431 A (SCRIPPS RESEARCH INST ;BARBAS CARLOS F III (US); GOTTESFELD JOEL M) 20 July 1995 see claims 1-52	32-38, 49-85, 88,90, 93,94
Y	WO 94 06814 A (GEN HOSPITAL CORP) 31 March 1994 see page 1, paragraph 3; claim 28	24
A	CROOK NE ET AL: "An apoptosis-inhibiting baculovirus gene with a zinc finger-like motif." J VIROL, APR 1993, 67 (4) P2168-74, UNITED STATES, XP000611841 cited in the application see the whole document	9-16, 25-38, 49-85, 88,90, 93,94
P,X	LISTON P ET AL: "Suppression of apoptosis in mammalian cells by NAIP and a related family of IAP genes." NATURE, JAN 25 1996, 379 (6563) P349-53, ENGLAND, XP002032296 see the whole document	1-4, 9-20, 25-31
P,X	DUCKETT CS ET AL: "A CONSERVED FAMILY OF CELLULAR GENES RELATED TO THE BACULOVIRUS IAP GENE AND ENCODING APOPTOSIS INHIBITORS" EMBO JOURNAL, 1996, 15, 2685-2694, XP002032297 see the whole document	1-4, 9-20, 25-31
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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

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INTERNATIONAL SEARCH REPORT

national application No.
PCT/IB 96/01022

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 39-48 and claims 34-38,85,88,90 partially as far as they concern an in vivo method, are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of that composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see continuation-sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

subject 1. (see continuation-sheet)

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/IB 96/ 01022

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

1.Claims 2-4 11 12 14-16 27 30 31 40 42 49 52 53 55-57 62-66 75 76 79 , all completely
claims 1 9 10 13 17-26 28 29 32-39 41 43-48 50 51 54 58-61 67-74 77 78 80-85 88 90-94, all partially
Apoptosis inhibiting proteins with ring zinc finger domains and with BIR (baculovirus IAP domain repeat) domains , their nucleic acids ,antibodies and antisense nucleic acids ,to be used in transgenic cells and animals containing them, in therapeutic compositions in methods to inhibit apoptosis in vitro ,methods to their identification and diagnosis

2.Claims 5-7 , all completely
claims 1 9 10 13 17-26 28 29 32-39 41 43-48 50 51 58-61 67-74 77 78 80-85 88 90-94, all partially
Apoptosis inhibiting proteins with no ring zinc finger domain and at least one BIR (baculovirus IAP domain repeat) domain , their nucleic acids , antibodies and antisense nucleic acids , to be used in transgenic cells and animals containing them ,in therapeutic compositions in methods to inhibit apoptosis in vitro , methods to their identification and diagnosis .

3.Claims 8 86 87 89 , all completely
claims 1 9 10 13 17-26 28 29 32-39 41 43-48 50 51 58-61 67-74 77 78 80-85 88 90-94, all partially
Apoptosis inhibiting proteins with a ring zinc finger domain and no BIR (baculovirus IAP domain repeat) domain , their nucleic acids ,antibodies , and antisense nucleic acids ,to be used in transgenic cells and animals containing them ,in therapeutic compositions in methods to inhibit apoptosis in vitro , methods to their identification and diagnosis .

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte onal Application No
PCT/IB 96/01022

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